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# PCBs (Polychlorinated Biphenyls) in Saginaw Bay: Development of Functional Indices to Estimate Inhibition of Ecosystem Fluxes

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# PCBS IN SAGINAW BAY: DEVELOPMENT OF FUNCTIONAL INDICES TO ESTIMATE INHIBITION OF ECOSYSTEM FLUXES

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16. ABSTRACT

Saginaw Bay is among the most polluted bays in the Great Lakes. For many years the Large Lakes Research Station of the US-EPA has examined many aspects of this ecosystem, from phytoplankton community characteristics to contaminant levels in fishes. As a result, when it became desirable to determine the impact of an organochlorine contaminant like PCB, it was not necessary to study the ecosystem in detail. Phytoplankton species and densities were known, zooplankton species, densities and feeding habits had been investigated.

This study produced new information on the two most basic fluxes in any aquatic system, the flow of solar energy into the phytoplankton, and the flow of solar energy into the phytoplankton, and the flow of chemical energy into the zooplankton. The use of phytoplankton gross photosynthesis to estimate the inhibition by contaminants of the first flux mentioned was developed for marine communities. In comparison, the use of zooplankton ingestion rates to estimate the inhibition by contaminants of carbon flow into secondary producers is new and developed for this investigation.

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Abstract: Aquatic ecosystems of the Great Lakes are threatened by approximately 50,000 chemicals of commerce from the United States and Canada; at least 2000 may have adverse biological effects and only 200 have been studied (IJC Annual Report, 1982). Contaminants as chlorinated hydrocarbons are especially prone to concentration in food chains because of their partitioning into lipids of lower organisms, and later food chain concentration by apex predators. Waters of the Great Lakes contain 2 to 300 ng $\ell^{1}$ PCB; this major contaminant is concentrated about 1 million times in apex predators. Generally the mode of action of PCBs is to inhibit organismic as well as ecosystem metabolism, and thus ecosystem productivity. Since the complex food webs of Lake Huron involve hundreds of phytoplankton taxa and tens of zooplankton taxa, we utilized and developed two ecosystem functional indices measuring contaminant inhibition. These were measures of the inhibition (and sometimes stimulation) of algal photosynthesis and of zooplankton grazing.

Methodololgically $^{14}C$-bicarbonate was used to measure phytoplankton gross photosynthesis. PCB concentrations of 5-500 ng $\ell^{-1}$ above control levels were injected into experimental test samples of phytoplankton incubated at depth of collection. Inhibition of photosynthesis was expressed as a percentage decrease relative to control values. This simple test is a functional (rate impacted) index of ecosystem inhibition at trophic level one (phytoplankton). In a similar treatment, $^{14}C$ tagged algae, fractionated into nannoplankton and netplankton size ranges (<20 µm and >20 µm dia.), were fed to zooplankton and filtering rates were calculated. Then similar tests were performed where experimental containers also contained PCBs. Thus the relative depression of grazing by PCBs was

measured for all major taxa of zooplankton.

Eight important conclusions were reached with regard to the Saginaw Bay ecosystem. With regard to phytoplankton photosynthesis, dichlorobiphenyl was selectively more toxic to nannoplankton than netplankton. This is an important conclusion, since Great Lakes food chains are based on small nannoplankton algae. Secondly, dichlorobiphenyl metabolites (unspecified) were more toxic to phytoplankton than the parent isomer. This finding suggested problems similar to those encountered for other man-made organics. In addition to selective toxicity by phytoplankton size, we found that PCBs inhibited photosynthesis of diatoms and green algae more than blue-green algae; unfortunately diatoms and small greens are the most grazed components in this ecosystem. Zooplankton grazing was also inhibited by PCB metabolites, and especially when detritus was present, as in most productive waters of the Great Lakes. The above findings were based on exposures to PCBs above normal environmental levels encountered in Lake Huron.

Inhibition of algal photosynthesis by ambient levels of PCBs was examined for dichlorobiphenyl and hexachlorobiphenyl and purified metabolites, including hydroxylated PCB and furans. Ambient levels of PCBs inhibited nannoplankton productivity. In the hexachlorobiphenyl series the hydroxylated PCB and furan were more inhibitory to photosynthesis than the parent isomer, with a seasonal range of inhibition of -2 to -93%. At depth these same contaminants stimulated gross photosynthesis, probably by increasing algal respiration and net production. This was a totally new result. In the dichlorobiphenyl series the metabolic products inhibited photosynthesis in surface waters from -6 to -22%, much less than by the higher chlorinated compound.

Lastly, we compared grazing in western Lake Erie to that in Saginaw Bay. In Lake Erie, grazing as a control on algal populations was almost as effective as in oligotrophic Lake Huron,, whereas grazing was greatly depressed in Saginaw Bay. Apparently the Lake Erie ecosystem is in much better condition than Saginaw Bay.

We conclude that functional ecosystem inhibition by toxic chemicals as PCBs is a most serious problem. Our results clearly indicate that levels of PCBs must be held below 5 ng $\ell^{-1}$ and we suggest that level as an appropriate water quality objective to the IJC. In addition, we suggest more attention be paid to the effects of PCB metabolites on natural communities. We also imply that the lack of zooplankton grazing in an ecosystem like Saginaw Bay is related to unknown inhibitory compounds with a mode of action similar or identical to PCBs.

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## SECTION 1

## INTRODUCTION

Aquatic ecosystems are by nature sensitive to contaminants which concentrate in food chains. The mechanisms involved in accumulation of polychlorinated biphenyls (PCB's) include physical concentration, equilibrium partitioning with lipoid materials (Pavlou and Dexter, 1977), and bioconcentration by higher organisms (many authors). These mechanissm take PCB's found in lakewater at concentrations in ppt (ng $l^{-1}$) and concentrate them until fishes often contain concentrations of ppm (mg $l^{-1}$).

The waters of the Great Lakes all contain about the same concentration of PCB's, with Lake Superior at about 2 ng $l^{-1}$ (Eisenreich, unpub.), Lake Michigan at 10 ng $l^{-1}$ offshore and 100 ng $l^{-1}$ near shore (Veith, 1975), Lake Huron at 5-316 ng $l^{-1}$ in Saginaw Bay (Richardson, pers. comm.), Lake Erie at 27 ng $l^{-1}$ and Lake Ontario at 30 ng $l^{-1}$ (Glooshenko et al., 1975). Thus concentrations in Great Lake waters generally fall within the same range and bear little resemblance to the degree of industrialization of surrounding countryside. However, Saginaw Bay, downriver from major industrial activity, is the largest input to Lake Huron (Frank et al., 1979). Dry deposition from the atmosphere accounts for a significant input of PCB's to the Great Lakes, along with the more obvious industrial dumping. Industrial manufacture of PCB's has been discontinued since 1972, but utilization of PCB's continues in liminted quantities. Aerosols containing PCB's may travel hundreds of kilometers (McClure, 1976) and constitute the principal input for lakes like Superior (Eisenreich

and Hollod, 1979), whose shores are not industrialized. Following dry deposition, PCB's may be concentrated in surface slicks or monolayers of lipoid materials (Duce et al., 1972). At the same time, losses of PCB's from surface slicks occurs through volatinzation (Southworth, 1979). Thus PCB's may be concentrated at the surface of the lakes, where biological uptake is most probable.

Phytoplankton accumulate PCB's, likely through equilibrium partitioning between water and lipid materials, although dead algae also concentrate them (Urey et al., 197 ). Equilibrium between algae and water occurs within two hours (Harding et al., 1978). Commonly literature values for the inhibition of carbon fixation by phytoplankton suggest a lower threshold of 10 $\mu g\ l^{-1}$ PCB (Harding, 1976; Powers et al., 1977), although Glooschenko et al. (1975) found 1 $\mu g\ l^{-1}$ inhibited photosynthesis in L. Ontario. This report will lower these thresholds considerably further.

Zooplankton reproduction (Daphnia) was affected by Aroclor (industrial PCB mixture) levels of 1.3 $\mu g\ l^{-1}$ (Nebeker and Puglisi, 1974). PCB's are commonly in equilibrium between the water and zooplankton lipids (Ware and Addeson, 1973; Pavlou and Dexter, 1977). Thus PCB's may be concentrated in marine zooplankton at levels of 0.3 to 260 $\mu g\ g^{-1}$ (Risebrough et al., 1972). Generally it has been concluded that ecological magnification increases with the degree of chlorination of the biphenyl (Metcalf, 1975).

PCB's are common in the fishes of Lake Huron, especially in herbivious alewives and predaceous walleyes and channel catfish (Reinert, 1970). Penta- and hexachlorobiphenyls are the most commonly found isomers in fishes (Ballschniter et al., 1978). Recently (Frank et al., 1978) PCB's were found in excess of 2 $\mu g\ g^{-1}$ in bloaters, splake, and cisco in Lake Huron. The white

sucker, an underutilized species of potential importance, contains 6-43 $\mu g\ g^{-1}$ in Lake Huron (Zabik et al., 1978).

At the top of the food chain, PCB's have been found in mother's blood at levels of 1.7-4.6 $\mu g\ l^{-1}$ (Kuwabara et al., 1979). Currently PCB's in Lake Ontario exceed International Joint Commission (IJC) water quality objectives and U.S.-E.P.A. criteria (Waller and Lee, 1979).

This brief introduction illustrates our considerable knowledge concerning the impact of PCB's on aquatic ecosystems. It also has revealed a distinct lack of knowledge in specific areas, to which this report is addressed. We know little of the impact upon a single simple food-chain, therefore we have chosen the approach of ecosystem analysis. We also know little of the effect of sure PCB isomers, as opposed to industrial Aroclors, on such food chains. More specifically we know very little concerning the impact of degradation products of PCB's on the biota of the Great Lakes. The effects of metabolites are critical to understanding total ecosystem inpact.

Generally the role of PCB's is to inhibit ecosystem metabolism and carbon flow. Other natural compounds also inhibit ecosystem functions. Inhibition of grazing by zooplankton was studied in Saginaw Bay and Lake Erie. Such inhibition in the natural environment is compared to that studied in the laboratory, where inhibition has been modeled (McMahon and Rigler, 1963) and where various biological factors resulting in inhibition of grazing have been studied in controlled experiments (Arnold, 1971; McNaught et al., 1980b).

The natural control of algal populations will be examined by looking for evidence of saturation feeding. In the oligotrophic waters of Southern Lake Huron saturation feeding does not occur; that is, ingestion increases linearly with food availability. During the periods of high phytoplankton growth, much

of the biomass is harvested. The grazing pressures were especially intense on the small nannoplankton (McNaught et al., 1980a). If zooplankton saturation feeding (inhibited ingestion) occurs in either Lake Erie or Saginaw Bay, these bodies of water are loosing important biological controls upon algal blooms.

## CONCLUSIONS

Fisrt we examined the impact of PCB's on the planktonic ecosystem of Saginaw Bay. A consideration of food chains in Lake Huron prompted the development of a simple *in situ* assay for both the acute toxicity and bioaccumulation of PCB's. Two trophic levels were considered, the first the primary producers, and the second, the primary consumers or herbivores, with the assay utilizing all of the natural biota of each trophic level. First a relatively large amount of $C^{14}$-bicarbonate was added to the natural phytoplankton assemblage to measure the rate of photosynthesis. After four hours incubation, the hot algae were fed to zooplankton, which were later assayed for $C^{14}$ activity. In a similar fashoning, $^{3}H$-PCB's were simultaneously added with $C^{14}$ to experimental phytoplankton samples. Thus the rate of uptake and standing-crop of PCB's could be measured; once these double-tagged algae had been fed to zooplankton, the uptake of PCB's and their bioaccumulation could be estimated. But most importantly, the effect of PCB's on carbon flow could be precisely measured. Our *in situ* assay considers the impact of PCB's on carbon flow through two trophic levels; the use of this technique led to eight important conclusions:

The parent isomer, 2, 2' PCB was more toxic to nannoplankton than netplankton. This is an important conclusion, since Great Lakes food-chains are based upon small nannoplankton algae. Secondly, based on exposure, 2, 2' PCB metabolites were more toxic than the parent isomer. Although estimates of metabolite abundance in the Great Lakes are not available, this finding suggests problems similar to those encountered for other man-made organics.

The greatest reductions of primary carbon fixation by PCB's were in the ecosystem contributions of diatoms and green algae, the most grazed-upon components. Sorption or binding was a significant factor in the accumulation of PCB's by these algae.

Surprisingly, reductions in grazing by zooplankton were also attributed to the presence of PCB metabolites. Again this finding points to potential widespread environmental problems; later we will show in an ecosystem naturally high in PCB's that little grazing occurs. The accumulation of PCB's by zooplankton was increased by the addition of detritus. We suspect that detritus binds PCB's and removes them from the dissolved pool; it also increases their availability to the higher trophic levels.

Secondly, we examined the inhibition (stimulation) of algal photosynthesis by low ambient concentrations of hexachlorobiphenyl and dichlorobiphenyl. The former was considered because of higher toxicity and slower degradability, while the latter degrades more rapidly. We asked whether ambient levels of 5 to 500 ng $l^{-1}$ were inhibitory. Both the intermediate hydroxylated and final degradation products (furans) were used, in addition to the parent isomer. The natural phytoplankton populations in Saginaw Bay were utilized for <u>in situ</u> estimates of productivity, with appropriate controls in all experimental series.

Ambient levels of PCB's (hexa- and dichlorobiphenyl) inhibited nannoplankton productivity. In the hexa- series the hydroxylated intermediates and the dibenzofuran were more inhibitory than the parent isomer in surface waters (seasonal range of inhibition of -2 to -93%). At depth, the parent hexachlorobiphenyl and degradation products stimulated carbon fixation (seasonal range or +7 to +57%). This is a new and striking result! Total average inhibition in the Saginaw Bay ecosystem is usually less than 10%, but may average 30% during periods of high spring runoff. The lower threshold for inhibition of photosyntheses was 5 ng $l^{-1}$ PCB, an order of magnitude lower than previously reported.

In the dichlorobiphenyl series, inhibition of photosynthesis occured in surface waters, where both the hydroxylated intermediate and furan reduced photosynthesis about 7% (range -6 to -22%), much less than that by the higher

chloronated isomer. Again, low ambient levels of dichlorobiphenyl stimulated carbon fixation at depth (seasonal range of +4 to +199%).

Light levels determined whether PCB's stimulated or inhibited algal photosynthesis. At depths (2.5-5 m) with 2-10% of surface light intensity PCB's were stimulatory. The level of chloronation was also a factor, as higher chloronation produced more inhibition. Thirdly, the inhibition of algal photosynthesis increased with the partition coefficients (log P) of the isomer. In this respect, the hydroxylated intermediate degradation products were much more toxic than their partition coefficients would indicate.

Thirdly, we examined the long term toxicity of PCB's to the crustacean Diaptomus. Natural exposure levels of 100 ng $l^{-1}$ to 10 µg $l^{-1}$ resulted in significant mortality in the laboratory. Such higher values are not encountered in Saginaw Bay.

Lastly, we considered natural zooplankton grazing as a control upon phytoplankton populations in Saginaw Bay and Western Lake Erie. In Saginaw Bay, some unknown environmental factor leads to significant inhibition of grazing. Populations of zooplankton consumed 86% less phytoplankton than they did in the open waters of Lake Huron. Such inhibition of grazing was not related to unusual abundances of blue-green algae. Since we have shown in this report that PCB's inhibit grazing in field situations, chemical inhibition is a possibility in Saginaw Bay. This is a serious environmental consequence, with obvious implications for reduced water quality.

In western Lake Erie, zooplankton grazing was only 37% less than levels observed in the open waters of Lake Huron. Apparently the Lake Erie ecosystem is in better shape than Saginaw Bay.

Ecosystem inhibition by toxic chemicals is a serious environmental problem. Our results indicate that levels of available PCB's should be held below 5 ng $l^{-1}$ (ppt) in Great Lakes waters. Natural levels of PCB's in Saginaw

Bay currently reach 316 ng $l^{-1}$. Since the degradation products of PCB's are so toxic at ambient levels, we suggest strongly that the USEPA fund additional studies concerning these compounds.

# SECTION 3

# RECOMMENDATIONS

## 3.1 RECOMMENDATIONS FOR WATER QUALITY CRITERIA ON PCB'S

The Toxic Substance Control Act has banned a well known and rightfully feared class of persistent toxic chemicals, the polychlorinated biphenyls. Considerable justification has been provided (Nisbet, 1977) for the establishment of a 1 ng $l^{-1}$ (1 ppt) level for natural waters. We must both agree and work to support this criteria, as we have shown (Section 5.2) a significant effect on photosynthesis upon exposure of phytoplankton to 5 ng $l^{-1}$. However, our most immediate responsibility is to protect the food fishes from contamination. Lake Huron, with 5-316 ng $l^{-1}$ in Saginaw Bay, produces fishes occupying lower trophic levels, as the alewife, with PCB levels of 0.1-0.6 ppm; more importantly, popular food fishes like the walleye, likely a component of trophic level five, are contaminated at unacceptably high levels (0.3-2.5 ppm), while the lake trout, the species considered by the International Joint Commission for revitalization in the upper lakes, is a very effective accumulator of PCB's, with levels of 0.2-1.8 ppm in Lake Superior. The splake, a lake trout x brook trout hybrid, had PCB levels of 0.3-1.5 ppm in Lake Huron, and the bloater, an oily species of chub, levels of 5-6.4 ppm (Frank et al., 1979). Clearly a cutoff of 25 ppt won't help, as shown by Lake Michigan. Logically 5 ppt PCB is a better objective, but levels below 1 ppt total PCB must be our ultimate goal.

## 3.2 RECOMMENDATION FOR FUTURE RESEARCH

The ecosystem approach must be utilized to a greater extent in testing persistent contaminants and their degradation products. Utilizing a single species of algae, as shown in many studies, gives very different response to a given exposure to PCB's than an intact phytoplankton community gives. The ecosystem approach utilizes the built-in interactions inherent to all ecosystems, whether the components tend to act synergistically or not. Moreover, in considering the ecysystem approach to an analysis of contaminant effects, the investigator is forced to involve related factors. In this particular study we discovered that PCB's stimulate carbon fixation at low light levels. We would not have made this discovery if we had grown a typical test algae in the presence of PCB's in a lighted growth chamber.

The U.S.-E.P.A. should provide support for studies of the degradation products of PCB's. We have shown that the intermediate hydroxylated products are more toxic than the chlorinated dibenzofurans. Yet we have no idea of the prevalence of such compounds in Lake Huron or any of the other Great Lakes. One of sciences saving graces is the ability of individuals to predict where problems will occur. From studies off DDT we realized that related degradation products behaved differently than the initial compound. Yet we have been slow to examine the environmental hazzards of other common metabolites.

Biologists studying the Great Lakes must consider the role of detritus in scrubbing toxic compounds from the water column, removing them from the pelagic food chain (and introducing them to the benthic food web). Lake Superior, with its continued atmospheric inputs of PCB's, may ultimately be more difficult to decontaminate because of its relatively low productivity of algae, and thus the low levels of particulate organics (detritus).

The waters of Saginaw Bay, with 5 - 316 ppt PCB, must be considered polluted. In effect, that pollution has created local investigations of which this is a component. Our findings regarding the effects of PCB's on the food chain in Saginaw Bay suggest that strong measures are required to limit inputs of PCB's to the Bay and ultimately the Lake. New creative measures to hold PCB's in river muds or to expedite their removal may be necessary.

A very basic biological problem common to all polluted ecosystems and all organisms must be tackled. We have examined the effects of PCB's on a few components of a natural system. Yet from these short-term experiments (4 hr or 30 days) we have no insight into the long-term adaptation of the plankton and fishes to PCB's. Short-term studies of physiological acclimation by phytoplankton and zooplankton to PCB's are needed; long-term studies of genetic adaptation are vital. It is certain that the phytoplankton, still abundant in Saginaw Bay, have adapted more rapidly to PCB's than humans have!

## SECTION 4

## EXPERIMENTAL PROCEDURES

### 4.1 METHODS FOR DETERMINING EFFECTS OF PCB'S ON THE SAGINAW BAY ECOSYSTEM

An abbreviated diagram of the ecosystem carbon flow experiment indicates the findings expected from the four treatments effecting both algal photosynthesis and zooplankton grazing (Table 1).

Phytoplankton methods

The natural assemblage of phytoplankton was harvested from 3 m depth with a pump. Polyethelene bottles of 1 l capacity were filled with lakewater and stored in the dark. All bottles were poured gently through 22 μm aperture nitex to separate out the netplankton, which were then resuspended in filtered lakewater. Controls were run upon and treatments applied to both nannoplankton and netplankton, which were then incubated *in situ* for 4 to 5 hr. about a time of midday.

The treatments were applied in the following fashion. An amount of a strong surfactant, Triton X-100, was added to appropriate bottles for a final concentration of 0.01%. This concentration of Triton alone was shown not to inhibit photosynthesis. Bottom sediments were harvested with a PONAR dredge; fine surface sediments were resuspended, allowed to settle, and the remaining fine sediments (200 ml) added to the phytoplankton. These treatments were applied following the addition of $^{14}$C-bicarbonate and preceeding the addition of $^{3}$H-PCB's.

Finally 2,2' dichlorobiphenyl in 3 ml acetone was added for a final con-

TABLE 1 . DESIGN OF EXPERIMENT TO OBSERVE EFFECTS OF PCB'S ON CARBON FLOW IN SAGINAW BAY ECOSYSTEM

| | Control and Treatments (4) | | | | |
|---|---|---|---|---|---|
| | Control: $^{14}C$ bicarbonate | Treatment 1: $^{14}C$ + $^{3}H$-PCB | Treatment 2: $^{14}C$ + $^{3}H$-PCB + surfactant | Treatment 3: + detritus | Treatment 4: $^{14}C$ + PCB metabolites |
| Phytoplankton | a) control upon primary productivity ($mgCm^{-3}$ $hr^{-1}$) | a) inhibition photosynthesis by PCB's | a) reduction in inhibition of photosynthesis | a) reduction in inhibition of photosynthesis by PCB's | a) inhibition of photosynthesis by metabolites |
| | b) accumulation PCB's by phytoplankton | b) reduction in PCB binding | b) particulate competition for PCB's | b) accumulation of metabolites by phytoplankton | |
| Zooplankton | a) control upon zooplankton ingestion | a) inhibition ingestion b. PCB's | a) reduced ingestion PCB's in food | a) reduced or increased PCB's in food | a) inhibition of ingestion by metabolites |
| | b) accumulation PCB's by zooplankton | b) reduced accumulation PCB's via food | b) reduced or increased accumulation of PCB's via foods | b) accumulation of metabolites by zooplankton | |

centration of 100 ng $l^{-1}$ or 25 µg $l^{-1}$. The PCB's were added following the addition of either the surfactant or the detritus. The 2,2' PCB was tritiated by New England Nuclear and had a specific activity of 2.0 mCi/mg. This provides appropriate counts from an aliquot of 100 ml of 100,000 to 400,000 dpm.

Following incubation for 4-5 hr, the samples were brought to the surface. Two subsamples of 100 ml each were filtered through glass fiber filters (GC), which were then ground in scintillation cocktail. The filter/cocktail solution was placed in a scintillation vial for counting in the laboratory.

In the laboratory the Beckman 200 scintillation counter was calibrated for best results in this double tag experiment. The efficiency of the counter was compared to the external standard ratio (ESR) measured with each counted sample. An appropriate $4^{th}$ order polynomial was fitted, using a stepwise regression analysis. In data reduction, corrected $^{14}C$ counts were calculated by selecting efficiencies indicated by appropriate ESR values.

Phytoplankton productivity was determined as outlined by Wetzel (1969).

Zooplankton methods

The natural assemblage of zooplantkon was collected with a 64 µm aperture net, vital to the inclusion of small herbivores like nauplii. These zooplankton were acclimated to ambient food concentrations for 2 hr. Then they were placed into the hot phytoplankton (with some phytoplankton foods treated by the 4 applications outlined). The zooplankton were allowed to feed for 15 min, following which they were filtered onto 2 coarse Whatman paper, narcotized with $CO_2$-water, and killed with hot water.

In the laboratory, 50 animals of each species were hand-picked and placed in scintillation vials. Usually four replicates of each common species were picked. The zooplankton were digested in Protosol at 53°C and

counted in the Beckman counter. Efficiencies for $^{14}C$ counting were determined as outlined above. Filtering rates were calculated according to the methods of Rigler (1971).

Preparation of metabolites

The $^{3}H$-2,2' dichlorobiphenyl was activated to the metabolite (likely a mixture of hydroxylated products and chlorinated dibenzofurans) by the use of the mixed microsomal function oxydose (MMFO) system of the rat liver (Shimada, 1976). We thank Jay Fisher and Ruth Anderson for preparation of these metabolites.

## 4.2 METHODS FOR DETERMINING EFFECTS OF PCB'S UPON CARBON FIXATION

Exposure levels of PCB's were matched to ambient levels in Saginaw Bay in an attempt to determine thresholds for inhibition of photosynthesis. Two isomers, dichlorobiphenyl and hexachlorobiphenyl were used, along with their probable degradation products.

Isomers and degradation products

The simulated hexachlorobiphenyl series tested in 1978 was obtained from the RFR Corp. and consisted of 2,2',4,4',5,5' hexachlorobiphenyl (RFR No. RPC 047), 2',3',3',3,3'-pentachloro-2-biphenol (RPM-25), and octachlorodibenzofuran (RPE-19). The simulated dichlorobiphenyl series tested in 1979 consisted of 2,2' dichlorobiphenyl (RPC-009), 4,4' dichloro-3,3' biphenydiol (RPM-14) and 2,8-dichlorobenzofuran (RPE-18).

Phytoplankton methods

Phytoplankton were collected at depths of 0.5, 2.5 and 5.0 meters by pump. The nannoplankton (< 22 µm diameter) were spearated from the netplankton (> 22 µm), which were resuspended in filtered lake water. Controls consisted of either sized plankton innoculated with 50 µCi $^{14}C$-bicarbonate and

incubated at the three depths of collection (total of 6 bottles). Experimental incubations of both nanno- and netplankton were treated with 5, 100 and 500 ng $l^{-1}$ of the parent isomer, and 100 ng $l^{-1}$ of the hydroxylated metabolite and the furan. The PCB's were dissolved in 1 ml of acetone and frozen prior to use. Thus 10 experimental incubations were made at each depth, for a total of 30 bottles.

Phytoplankton controls and treatments were incubated for 4 hr in situ. After that time the bottles were brought to the surface. Two 100 ml subsamples were taken from each of 36 bottles and filtered through a glass fiber (GC) filter, which was then ground in scintillation cocktail. The ground filter was diluted with cocktail to a constant volume in a scintillation vial and returned to the laboratory for counting. All work with PCB's was conducted by investigators wearing face masks (respirometers).

Calculations of phytoplankton productivity were made using standard procedures (Wetzel, 1969) following scintillation counting in the laboratory. All counts were corrected for counting efficiency.

Light measurements

Measurements of light intensity at depth were made with a Montodoro (LMT-8A) light meter. Readings were expressed as a percentage of sun's intensity in late June (at maximum azimuth). This gave us a measure of relative intensity over a growing season, as compared to the commonly employed % surface intensity.

## 4.3 METHODS FOR DETERMINING EFFECTS OF CHRONIC EXPOSURE TO PCB'S

Experimental procedures

Zooplankton from Lake Huron via the Detroit River were collected in a net of 64 µm aperture and brought into the laboratory. Ten zooplankton of the

genus Diaptomus were handpicked and placed into glass tissue culture bottles containing filtered river water. These culture bottles were then corked and attached to silicon tubing from a manostatic pump. The pump drew water from four stock bottles containing three concentrations of dichlorobiphenyl (100 ng $l^{-1}$, 1 µg $l^{-1}$, and 10 µg $l^{-1}$), and the control water. Five culture flasks containing 10 zooplankton each served as controls. Each of three concentrations of PCB's were pumped through 5 additional flasks, to make a total of 20 flasks containing 200 animals per experiment. The rate of pumping of PCB's could be varied from 0-9 ml $hr^{-1}$; we selected 1 ml $hr^{-1}$.

Twice each day the 20 culture flasks were examined with a hand lens to determine the number of animals alive (swimming) and those values recorded in tabular form, as summarized in the results.

Problems with procedure

The obvious lack of effect of 2,2' dichlorobiphenyl on the survivorship of Diaptomus prompted a careful analysis of the concentrations of dichlorobiphenyl reaching the culture flasks.

In the experiment of 12 March 1979, the stock bottles were teflon and silicon tubing was used to carry water to the culture flasks. The concentration of PCB in the more dilute stock bottle was expected to be 3.43 µg $l^{-1}$; it was never observed above detectable limits. The concentration of PCB in the more concentrated bottle was expected to be 200 µg $l^{-1}$; it varied between 0 and 27 µg $l^{-1}$. Obvious problems existed in maintaining desired concentrations of PCB.

Therefore in planning the experiment of 24 March 1980, we used glass stock bottles and tubing, with the employment of silicon tubing only within the pump itself. Over a period of days 0 to 7, the PCB concentration in the

bottle expected to be 3.8 μg $l^{-1}$ varied from 3.0 to 0.3, with a regularly decreasing concentration. The concentration in the 20 μg $l^{-1}$ stock bottle varied from a high of 7.4 μg $l^{-1}$ on day 1 to a low of 1.7 μg $l^{-1}$ on day 7.

Because of severe problems regarding the concentrations of PCB's to which organisms were subjected, we will limit the presentation and discussion of these data.

## 4.4 METHODS FOR ASSESSING ZOOPLANKTON GRAZING

For total grazing a modification was used of the original methods of Nauwerck, who determined grazing using tagged ($^{14}C$) phytoplankton. The radioactivity of zooplankton was measured, after a brief feeding period (15 min), too short for the production of radioactive faeces at low food levels. Before being given radioactive food, the animals were acclimated for 1 hr in non-radioactive cells of the same natural assemblage tagged with $Na^{14}CO_3$. Approximately 2000 animals were acclimated in 600 ml of natural algae in lakewater. After 1 hr, 400 ml of tagged food was added. The animals were then allowed to feed on the hot mixture for 15-30 min. They were relaxed with $CO_2$ water, killed with boiling water, and preserved. Each experiment was run in quadruplicate. In the laboratory animals were picked, using a 1 mm diameter metal loop, carefully avoiding the removal of large filamentous algae. Approximately 50 animals were placed in a scintillation vial and digested with Protosol (New England Nuclear); after 24 hr at 60°C, the vials were filled with 10 ml of cocktail. All samples were placed in the dark for 24 hr to eliminate chemoluminescence, and then counted in a Beckman Model 133 liquid scintillation counter. Typically zooplankton samples were counted with an efficiency of 95%, as contrasted to phytoplankton foods counted at 65% efficiency.

For selective grazing, techniques similar to the above were employed, except that food resources were size-fractionated. The natural assemblage of phytoplankton was passed through a fine net (22 μm), leaving the nannoplankton (< 22 μm) in the lakewater and removing the netplankton (> 22 μm), which was resuspended in filtered (0.45 μm) lakewater. Tagged ($^{14}C$) nannoplankton, following a 4 hr in situ incubation, were mixed with cold netplankton to reconstitute the natural assemblage. In a similar manner, tagged netplankton were mixed with cold nannoplankton just before feeding. The natural assemblage of herbivores, already acclimated to their food assemblage, was reacclimated for 1 hr in water-cooled shipboard tanks. Animals accliminated in 400 ml lakewater were fed 600 ml hot food. After feeding for 12-15 min the animals were killed and preserved.

Assimilation was calculated by feeding animals for 2 hr, as above, and then removing them and placing them in cold food for 4 hr, during which time any hot food would be eliminated from the gut. Assimilated food was measured as above, using liquid scintillation techniques. Percentage assimilation (Sorokin, 1968) was calcualted from the ratio mgC assimilated $hr^{-1}$:mgC ingested $hr^{-1}$.

## 4.5 LOCATION OF EXPERIMENTS

### Saginaw Bay, Lake Huron

The effect of algal productivity on zooplankton grazing was examined on Saginaw Bay during 1976, while the effects of PCB's on food chain dynamics were studied from 1977 through 1979. In 1976 all feeding experiments were conducted in Segment III at Station 56, west of Fish Point (Figure 1). In 1977 the effects of PCB's on phytoplankton photosynthesis and zooplankton ingestion were measured at the same station. During 1978 and 1979 the effects of low (ambient) concentrations of PCB's were usually studied at Station 56, but in both years strong September storms forced skipper Ed McHugh and crew to seek the shelter of the leeward shore at Station 2, southeast of Nayanquing Point. Station 56 was originally selected because it was characterized by the highest algal productivity in the Bay.

### Western Lake Erie

The effect of algal productivity on zooplankton grazing was likewise examined in Lake Erie. The Ohio State University motor vessel Hydra, under the leadership of Denny Naylon was anchored for all studies at Station 59, just 2 miles SW of West Sister Island. In times of high westerly winds, the vessel was moved to Station 59 North in the leeward of West Sister Island.

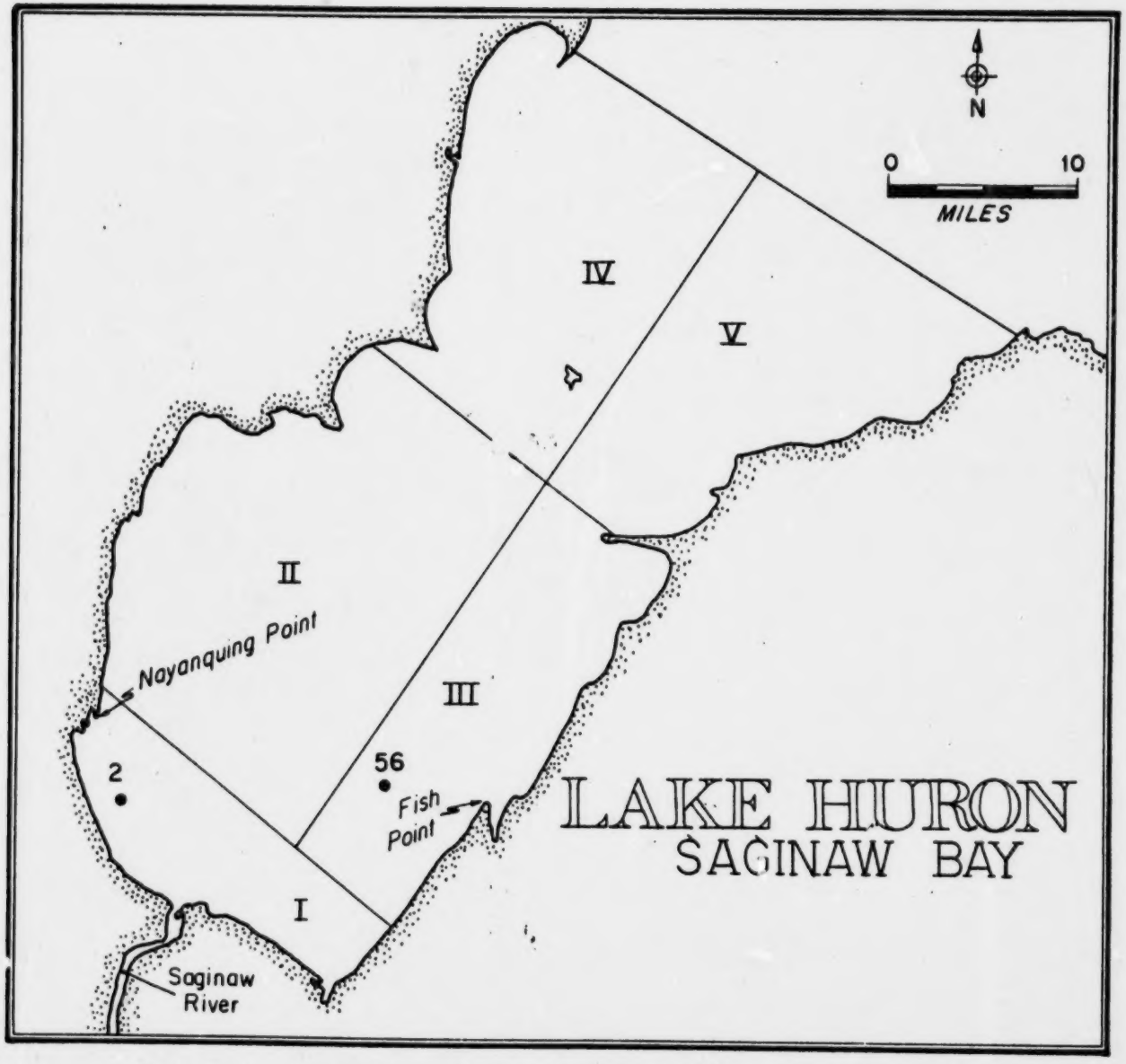


Figure 1. Location of studies on Saginaw Bay, Lake Huron.

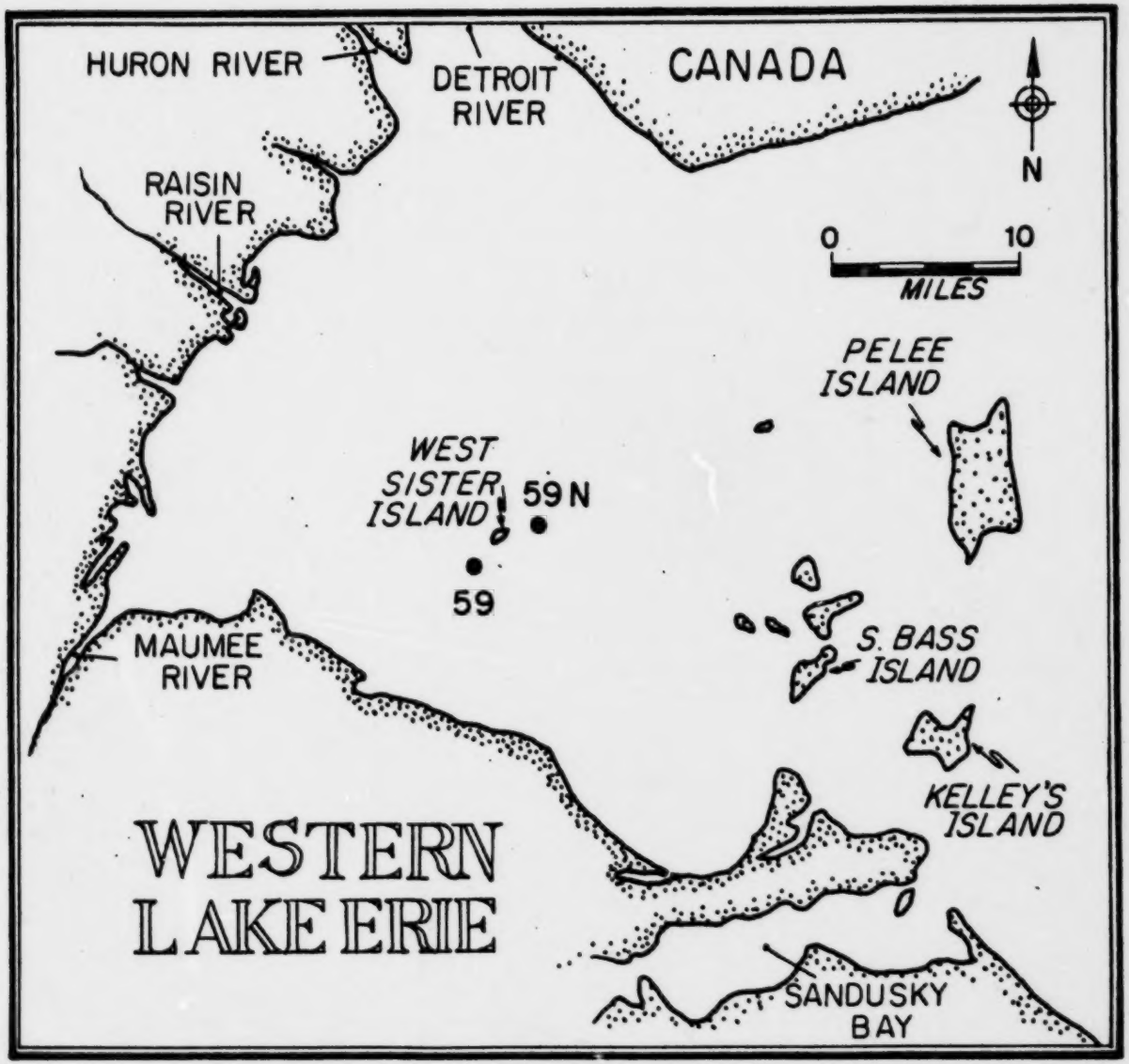


Figure 2. Location of studies on Western Lake Erie.

## 5.1 EFFECTS OF PCB'S UPON CARBON FLOW IN THE SAGINAW BAY ECOSYSTEM

### Purpose

The upper, food producing levels of aquatic ecosystems in the Great Lakes are based upon planktonic food chains. The basic processes of primary productivity by phytoplankton and grazing by zooplankton may be impared by toxic substances. We proposed to examine the important fluxes and standing crops of the Huron ecosystem. A controlled experiment, utilizing tagged PCB's in various treatments, was designed to meet our six basic purposes including:

1. to measure the effects of PCB's on carbon fixation by phytoplankton,
2. to measure the effects of PCB's on the grazing rates of zooplankton; in addition, to examine the resultant accumulation of PCB's in zooplankton,
3. to estimate the relative role of adsorption (binding) in the concentration of PCB's at upper trophic levels,
4. to estimate the role of detritus in concentration PCB's in the food chain,
5. to measure the inhibition of the above fluxes of carbon (to algae and zooplankton by PCB metabolites,
6. to examine the seasonal effects of PCB's on the algae (selective inhibition of major groups of algae).

### Detailed Results of Field Experiments

Experiment of 28 May 1977--

Phytoplankton productivity was markedly reduced by PCB's. Phytoplankton populations were dominated by diatoms (94% biovolume). The important genera were Asterionella formosa, Cyclotella, Diatoma, Melosira islandica, Stephano-

discus binderanus, and Tabellaria fenestrata. The non-diatoms were rare, and consisted of Cryptomonas orata and Dinobryon divergans (2.8% biovolume) and bluegreens (2.1%) (pers. comm., E. Stoermer).

In this initial experiment, phytoplankton populations received a treatment of 100 ng $l^{-1}$, 2,2'dichlorobiphenyl. The rate of carbon fixation by the nannoplankton (< 22 µm diameter) was reduced from 3.72 $mgCm^{-3}$ $hr^{-1}$ to 1.78, a reduction of 52%. With the addition of a surfactant (0.01% Triton), carbon fixation was reduced to 2.48 $mgCm^{-3}$ $hr^{-1}$, a reduction of only 33%. Thus bnding of PCB's was an important mechanism in the inhibition of nannoplankton photosynthesis. At the same time the netplankton productivity was not effected (Table 2).

Grazing by zooplankton was not reduced significantly by the addition of 100 ng $l^{-1}$ dichlorobiphenyl (Table 3). Copepod nauplii and copepodites, as well as adult Chydorus sphaericus, failed to exhibit significant reductions in filtering rates in the presence of PCB's. This conclusion applies to both nannoplankton and netplankton foods.

However, the addition of organic detritus caused the same animals to filter more rapidly. Thus detritus was likely a preferred food; it also carries adsorbed PCB and should significantly increase the intake of PCB's by these animals (Table 4).

Experiment of 9 July 1977--

The treatment levels with PCB's and metabolites were increased to 25 µg $l^{-1}$ to follow the effects on the zooplankton more closely. In addition, the PCB's were tritiated, utilizing the double tag ($C^{14}$ and $H^{3}$) technique for the first time in this study.

Phytoplankton populations were comprised of greens (44.3% biovolume),

TABLE 2 . EFFECT ON PHYTOSYNTHETIC RATES ($mgCm^{-3}$ $hr^{-1}$) OF NATURAL NANNOPHYTOPLANKTON (< 22 μ) AND NETPHYTOPLANKTON (> 22 μ) POPULATIONS OF ADDITON OF 100 ng $l^{-1}$ 2-2' DICHLOROBIPHENYL. MAY 28, 1977 AT STATION S56, SAGINAW BAY, LAKE HURON

| | Population Size Class | | |
|---|---|---|---|
| Treatment | Nannoplankton | Netplankton | Total |
| Control | 3.72 ± .09 | 1.76 ± .14 | 5.48 |
| 100 ng $l^{-1}$ 2-2' PCB | 1.78 ± .12 | 1.86 ± .16 | 3.64 |
| 100 ng $l^{-1}$ 2-2' PCB + 0.01% Triton | 2.48 ± .06 | 1.38 ± .05 | 3.86 |

TABLE 3 . FILTRATION RATES (ml $an^{-1}$ $hr^{-1}$) OF DOMINANT SPECIES OF ZOOPLANKTON FED NATURAL POPULATIONS OF PHYTOPLANKTON GROWN IN 100 ng $1^{-1}$ DICHLOROBIPHENYL, 28-29 MAY 1977

| | Filtering Rates Upon | | | | |
|---|---|---|---|---|---|
| | Nannoplankton (<22μ) | | Netplankton (>22μ) | | |
| Species or Life Stage | Control | with 100 ppt PCB | Control | with 100 ng $1^{-1}$ PCB | with 100 ng $1^{-1}$ PCB+ detritus |
| Copepods | | | | | |
| Copepod nauplii | 0.002±0 | 0.004±0.002 | 0.005±0.001 | 0.004±0 | 0.024 |
| Cyclopoid copepodites | 0.002±0.001 | 0.004±0.001 | 0.007±0.001 | 0.004±0.001 | 0.027 |
| Cyclops bicuspidatus(A) | 0.006±0.001 | - | 0.003±0.001 | - | - |
| Cladocerans | | | | | |
| Chydorus sphaericus | 0.004±0.001 | 0.005±0.001 | 0.008±0.002 | 0.005±0.001 | 0.025 |

bluegreens (33.0%), flagellates (6%), and diatoms (16%).

Carbon fixation by the phytoplankton was reduced 68% with the addition of 25 µg $l^{-1}$ dichlorobiphenyl. The fixation by nannoplankton was reduced 53%, whereas the netplankton was reduced 74.6% (Table 4).

When the surfactant was added, the fixation by nannoplankton was reduced only 36.1% by PCB's, as opposed to 53% without, a significant alleviation of both binding and inhibition. Inhibition of nannoplankton algae by PCB's may thus be related to surface binding. However, the net algae behaved differently. Fixation of carbon was reduced 74.6% without the surfactant, and 68% with the addition. It appeared that binding was not so important a factor in concentrating PCB's in the case of net algae, many of which were bluegreens (Table 4).

PCB metabolites had a very inhibitory effect on carbon fixation. Separate tests were not performed on the different size classes of algae. However, total carbon fixation was reduced from 5.54 $mgCm^{-3}$ $hr^{-1}$ to 0.7, a reduction (inhibition) of 87.3% (Table 4). Obviously metabolites of PCB's are more toxic than the parent isomer.

Accumulation rates by algae of dichlorobiphenyl were also estimated, using the $H^3$ tag. The nannoplankton accumulated 0.013% $hr^{-1}$ of their dry weight in PCB's, whereas the netplankton accumulated 0.079% $hr^{-1}$. The surfactant did not reduce accumulation rates (Table 5). We thus see that while nannoplankton, the base of the food chain, accumulate less the impact on carbon fixation is very great. Indeed the nannoplankton are very sensitive to PCB's.

The effect of PCB's on the filtering rate of zooplankton of 9 July was not significant. The zooplankton filtered just as much algae in the presence

TABLE 4. EFFECT ON PHOTOSYNTHETIC RATES ($mgCm^{-3}\ hr^{-1}$) OF NATURAL NANNOPLANKTON (< 22 µm) AND NETPLANKTON (> 22 µm) OF 25 $\mu g\ l^{-1}$ 2-2' DICHLOROBIPHENYL. JULY 9, 1977 AT STATION S56, SAGINAW BAY, LAKE HURON

| | Population Size Class | | |
|---|---|---|---|
| Treatment | Nannoplankton | Netplankton | Total |
| Control | 1.63 ± .10 | 3.91 ± .19 | 5.54 |
| 25 ppb 2-2' PCB | .77 ± .05 | .99 ± .08 | 1.76 |
| 25 ppb 2-2' PCB + 0.01% Triton | 1.34 ± .07 | 1.30 ± .05 | 2.64 |
| 25 ppb 2-2' PCB metabolites | --- | --- | 0.7 |

TABLE 5 . AMOUNT (% DRY WT $HR^{-1} \times 10^2$) OF 2-2' PCB (25 $\mu g\ l^{-1}$) ACCUMULATED BY ALGAE DURING 4 HR INCUBATION. JULY 9, 1977 SAGINAW BAY, LAKE HURON

| | Algae Size Class | | |
|---|---|---|---|
| Treatment | Nannoplankton | Netplankton | Total |
| 25 ppb 2-2' PCB | 1.31 | 7.92 | 2.52 |
| 25 ppb 2-2' PCB + 0.01% Triton | 1.61 | 8.74 | 2.91 |

of PCB's as they did in natural waters (Table 4 ). However, this does not mean that PCB's do not effect the zooplankton, as we will discuss later.

Experiment of 28 July 1977--

Phytoplankton populations were dominants in Saginaw Bay in late July by green algae (73.5% biovolume) including Oocystis and Cosmarium; bluegreens (14.0%) including Chroococcus; flagellates (8.9%), mainly Dinobryon; and diatoms (3.6%), including Asterionella, Surinella and Cyclotella (Stoermer, pers. comm.).

The total carbon fixation rate on 28 July was 10.16 $mgCm^{-3}$ $hr^{-1}$. Dichlorobiphenyl (25 $\mu g$ $l^{-1}$) reduced the carbon fixation to 3.12, an inhibition of 69%. The nannoplankton photosynthesis, being more sensitive to PCB's, was reduced 77% and the netplankton 64% (Table 6 ).

Adsorption of PCB's was again important. Treatment with PCB's and the surfactant reduced carbon fixation to 5.42, only 42%, as opposed to the 69% reduction without surfactant.

PCB metabolites reduced carbon fixation from 10.16 $mgCm^{-3}$ $hr^{-1}$ to 1.63, a significant inhibition of 84% (Table 6 ).

The accumulation of PCB's by the phytoplankton again told an important story. PCB metabolites accumulated more rapidly than the parent dichlorobiphenyl; the rate of metabolite accumulation (.093 d.w. $hr^{-1}$) was 4.3 times faster than the rate of accumulation of the parent isomer (0.22 $hr^{-1}$). This high rate of accumulation of metabolites by algae (Table 7 ) may be directly related to the high level of inhibition of carbon fixation by metabolites.

The parent 2,2' PCB isomer had little effect upon zooplankton filtering rates. In contrast the metabolites (25 $\mu g$ $l^{-1}$) inhibited feeding by 43%. Species affected by the parent isomer included only Daphnia retrocurva and

TABLE 6. EFFECT ON PHOTOSYNTHETIC RATES ($mgCm^{-3}$ $hr^{-1}$) OF NATURAL NANNOPLANKTON (< 22 μm) AND NETPLANKTON (> 22 μm) OF 25 μg $l^{-1}$ 2-2' DICHLOROBIPHENYL. JULY 28, 1977, SAGINAW BAY

| | Population Size Class | | |
|---|---|---|---|
| Treatment | Nannoplankton | Netplankton | Total |
| Control | 3.65 ± .62 | 6.51 ± .89 | 10.16 |
| 25 ppb 2-2' PCB | 0.81 ± .23 | 2.31 ± .26 | 3.12 |
| 25 ppb 2-2' PCB + 0.01% Triton | 2.36 ± .31 | 3.06 ± .22 | 5.42 |
| 25 ppb 2-2' PCB metabolites | --- | --- | 1.63 ± .20 |

TABLE 7. AMOUNT (% DRY WT $HR^{-1}$ x $10^{-2}$) OF 2-2' PCB (25 μg $l^{-1}$) ACCUMULATED BY ALGAE DURING 4 HR INCUBATION. JULY 9, 1977, SAGINAW BAY

| | Algae Size Class | | |
|---|---|---|---|
| Treatment | Nannoplankton | Netplankton | Total |
| 25 ppb 2-2' PCB | 1.4 | 4.92 | 2.15 |
| 25 ppb 2-2' PCB + 0.01% Triton | 3.68 | 6.96 | 4.62 |
| 25 ppb 2-2' PCB metabolites | ---- | ---- | 9.34 |

Cyclops vernalis (Table 8 ). Species whose filtering rates were inhibited an average of 47% by the metabolites of dichlorobiphenyl included Chydorus sphaericus, Daphnia retrocurva, Eubosmina coregoni and copepod nauplii, the most important grazers in this ecosystem (Table 8 ).

Accumulation rates of 2,2' PCB and two mixed metabolites by zooplankton were rapid. Five of seven species or stages feeding upon nannoplankton had rates of accumulation of 0.002 to 0.006% d.w. per 15 min. exposure (Table 9 ). The highest rate of accumulation was by nauplii, the most active feeders in Lake Huron (McNaught et al., 1980). Two species of Cyclops, and Chydorus and Daphnia also had high rates of accumulation when feeding on nannoplankton.

When detritus was added to nannoplankton incubated in PCB's and then fed to zooplankton, the rates of accumulation of PCB's increased in five of seven cases (Table 9 ). It would appear that many zooplankton which graze on algae feed simultaneously upon detritus. In productive environments detritus is abundant, adsorbs PCB's, and thus serves to increase the PCB intake of herbivorous organisms.

Ecosystem Impact of PCB Acute Toxicity

Experiment of 28 July 1977--

The experimental addition of 25 µg $l^{-1}$ 2,2' PCB to the Saginaw Bay ecosystem had important short-term consequences (Figure 3 ). In this diagram the flow of carbon is diagrammed with arrows and boxes and summarized on the right side, while the effect of PCB's upon carbon flow is summarized on the left.

Fluxes of carbon into the phytoplankton (controls) were reduced by 2,2' PCB (-69%), alleviated by surfactants, and inhibited more severely by metabolites (-84%). Bioaccumulation of $H^3$-PCB by the phytoplankton was significant

TABLE 8. COMPARISON OF FILTERING RATES FOR ZOOPLANKTON FEEDING ON NANNOPLANKTON POPULATIONS GROWN AS CONTROLS, IN 25 µg $l^{-1}$ DICHLOROBIPHENYL, AND IN 25 µg $l^{-1}$ METABOLITES, JULY 28, 1977

| | Filtering Rates (ml $an^{-1}$ $hr^{-1}$) | | |
|---|---|---|---|
| Species | Control | Dichlorobiphenyl | Metabolites |
| Chydorus sphaericus | .064 ± .031 | .041 ± .017 | .028 |
| Daphnia retrocurva | .138 ± .046 | .254 ± .008 | .127 |
| Eubosmina coregoni | .071 ± .032 | .038 ± .034 | .026 |
| Nauplii | .106 ± .009 | .099 | .049 |
| Cyclops vernalis | .126 ± .063 | .272 ± .039 | ---- |
| Tropocyclops prasinus | .061 ± .031 | --- | ---- |
| Diaptomus spp. | .087 ± .051 | --- | ---- |

TABLE 9. AMOUNT OF 2-2' PCB (25 μg $l^{-1}$) ACCUMULATED (μg/g) IN 15 MIN. FEEDING ON NANNOPLANKTON (<22μm) AND NETPLANKTON (>22μm) WITH REGARD TO TREATMENT, JULY 28, 1977.

| | Nannoplankton plus | | | Netplankton plus | | |
|---|---|---|---|---|---|---|
| Species or Stage | PCB | PCB+Triton | PCB+Detritus | PCB | PCB+Triton | PCB+Detritus |
| Chydorus sphaericus | 20.7 | 49.2 | 73.7 | 95.9 | 68.1 | 1.6 |
| Cyclops vernalis | 33.6 | 34.1 | 55.9 | 18.0 | 51.8 | 13.4 |
| Daphnia retrocurva | 22.9 | 27.5 | 24.9 | 13.5 | 32.2 | 11.0 |
| Eubosmina coregoni | 8.9 | 43.0 | 15.2 | -- | 12.6 | 7.0 |
| Nauplii | 60.3 | 84.9 | 144.9 | 10.7 | 53.4 | 86.8 |
| Cyclops bicuspidatus | 14.6 | 19.4 | 34.1 | 10.9 | 26.9 | 0.2 |
| Diaptomus spp. (A) | 7.5 | 16.7 | 28.6 | 29.7 | 26.0 | 1.7 |
| Mean | 24.1 | 39.3 | 53.9 | 29.8 | 38.7 | 17.4 |

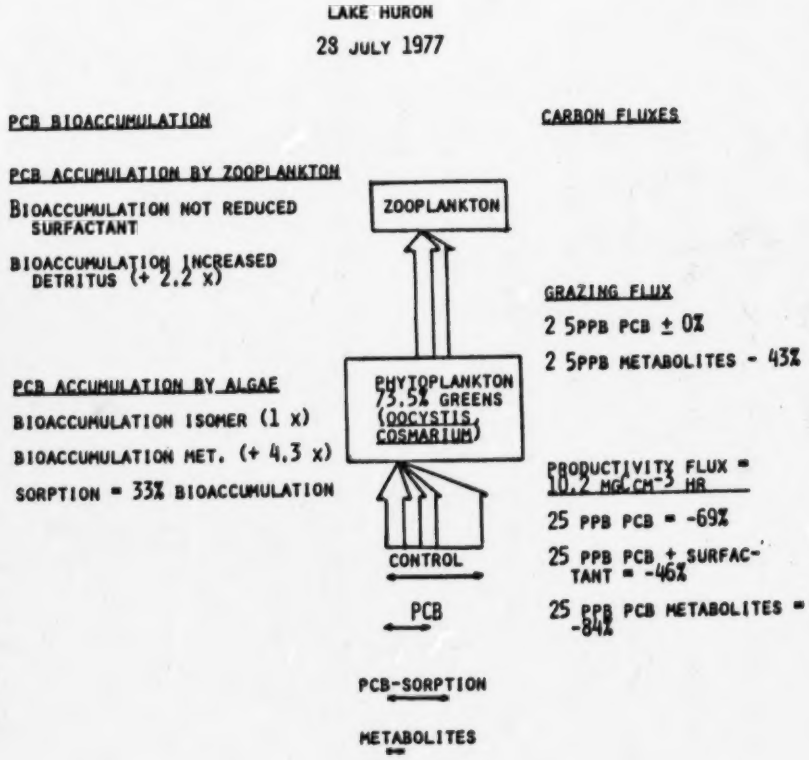


Figure 3. Ecosystem impact of experimental addition of 25 μg $l^{-1}$ dichlorobiphenyl, including inhibition of carbon flow (right column) and accumulation of PCB with trophic levels (left column).

with the relative bioaccumulation noted (1X). The bioaccumulation of PCB metabolites exceeded that of the parent isomer by a factor of 4.3 X.

Zocplankton grazed upon these experimental phytoplankton populations, in this instance composed of the green algae Oocystis and Cosmarium. The grazing rate of the zooplankton ( controls ) was not reduced by 25 µg $l^{-1}$, 2,2' PCB. This was not a fortunate circumstance as it might seem, since they therefore ingested PCB rich algae and then bioaccumulated PCB. However, the PCB metabolites were apparently acutely toxic to zooplankton, inhibiting ingestion of phytoplankton by 43%. The ultimate bioaccumulation of 2,2' PCB by the zooplankton was not significantly affected by reducing adsorption of PCB's on algae. Bioaccumulation of 2,2' PCB by zooplankton was increased when detritus was added to the nannoplankton foods (Figure 3 ).

Relative Toxicity of 2,2' PCB and Metabolites to Phytoplankton

The relative toxicity of 2,2' PCB and its metabolites to phytoplankton must be determined relative to the dose received, and not simply the environmental exposure. Th exposure was determined from the amount of $H^3$-PCB added to the phytoplankton cultures. The dose was calculated from $H^3$ uptake, and the relative acute toxicity of 2,2' PCB from phytoplankton carbon fixation relative to the photosynthesis in the control cultures.

The effect of 25 µg $l^{-1}$ of the parent 2,2' PCB upon nanno- and netplankton, as well as the effect of the metabolites on the total phytoplankton assemblage is summarized for the cruises of 9 July and 28 July 1977 (Table 10). The inhibitory effect of the PCB on photosynthesis (% dec) divided by the dose (PCB accumulation in 4 hr) gives an estimate of relative toxicity. Based on dose, the parent isomer of 2,2' PCB is more toxic to the nannoplankton (40.5, 55.0) than the netplankton (9.3, 13.1) by a factor fo 4.3 X. The

TABLE 10. RELATIVE LEVELS OF TOXICITY OF PARENT PCB ISOMER (2-2') AND PCB METABOLITES TO SMALL AND LARGE ALGAE

| Size Phytoplankton | PCB Product | % Decrease in Photosynthesis Rate | PCB Accumulation (%d.w./4hrs) | Relative Toxicity (%dec/%d.w.) |
|---|---|---|---|---|
| nanno (< 22 µm) | Parent Isomer | 53 | $1.31 \times 10^2$ | 40.5 |
| nanno (< 22 µm) | Parent Isomer | 77 | 1.40 | 55.0 |
| net (> 22 µm) | Parent Isomer | 74 | 7.92 | 9.3 |
| net (> 22 µm) | Parent Isomer | 64 | 4.90 | 13.1 |
| total (all sizes) | Metabolites | 84 | 9.30 | 9.0 |

TABLE 11. MECHANISM OF PHOTOSYNTHETIC AFFECT. SORPTION OF PCB'S ON CELL WALL INVOLVED IF SURFACTANT INCREASES PHOTOSYNTHESIS ABOVE LEVEL OF TREATMENT (PCB)

| Size Phytoplankton | Increase in Photosynthesis PCB & Surfactant | Forms |
|---|---|---|
| nanno (< 22 µm) | 36% | small diatoms |
| nanno (< 22 µm) | 42% | small diatoms |
| net (> 22 µm) | 8% | large bluegreens |
| net (> 22 µm) | 0% | large bluegreens |

metabolites are less toxic than the parent isomer per unit dose, but in field experiments more metabolites are accumulated, accounting for greater decreases in photosynthesis at constant levels of exposure. It is expected that the metabolites bind more actively than the parent isomer.

Mechanisms of Algal Inhibition

Field studies suggest that phytoplankton taxa adsorb PCB's to different degrees. If we select field data illustrating inhibition of carbon fixation, wherein the phytoplankton in a given size group (nanno- or netplankton) are of chiefly one order, we gain insight into one possible mechanism.

Data from two months are presented (Table 11). The nannoplankton were predominantly small diatoms and greens, whereas the netplankton were composed of greens.

The experiment is interpreted as follows. In all cases the addition of dichlorobiphenyl (25 $\mu g\ l^{-1}$) inhibited photosynthesis. In parallel experimental series, the addition of a surfactant with the PCB either again increased photosynthesis, or affected the PCB treatment very little. Where diatoms and greens were involved, the addition of a surfactant with the PCB led to an alleviation of inhibition. Thus we interpret these results to mean that binding to cell walls/membranes was reduced. Where bluegreens were involved, the addition of a surfactant with the PCB did not increase photosynthesis. Bluegreens are surrounding with a hydrophilic sheath which may interfere with binding PCB's. Thus bluegreens may not initially suffer from PCB's to the degree which diatoms and greens do. Bluegreens and other sheathed forms may have a selective advantage in environments polluted with PCB's.

Summary of Effects of PCB's on Carbon Flow

1. The parent isomer, 2,2' PCB was more toxic (inhibitory to carbon fixation) to nannoplankton than netplankton.
2. On the basis of exposure, PCB metabolites were more toxic than the parent 2,2' PCB.
3. The greatest reductions by PCB's were in photosynthetic rates of diatoms and green algae.
4. Sorption or binding was a significant factor in the accumulation of PCB's by phytoplankton (excluding bluegreens).
5. Zooplankton feeding rates were not affected by 2,2' PCB.
6. Zooplankton feeding rates were reduced by 2,2 PCB metabolites.
7. The accumulation of PCB's in zooplankton was increased through the addition of detritus.
8. In an evolutionary sense, PCB's may increase the fitness of bluegreens at the expense of diatoms.

## 5.2 INHIBITION OF NATURAL PHYTOPLANKTON PHOTOSYNTHESIS AT ENVIRONMENTAL CONCONCENTRATIONS OF PCB'S AND METABOLITES

### Purpose

PCB's are present in the waters of Saginaw Bay (Station 56), Lake Huron, at concentrations of 5-316 ng $l^{-1}$ (Table12). We originally proposed to examine the inhibitory effects of PCB's and their degradation products upon algal photosynthesis. In the process of this investigation, as clearly shown in the results which follow, we made the important discovery that PCB's also stimulate carbon fixation by phytoplankton. These two specific implications are examined for natural mixed populations of nannoplankton (> 22 µm diameter) and netplankton (> 22 µm diameter).

### Description of Phytoplankton Populations

During the summer of 1978 diatoms constituted 1.4 to 43.8% of the nannoplanktic biovolume in Saginaw Bay (Table13). Melosira was far the dominant genus. In contrast, non-diatoms made up 56.2 to 98.6% of the nannoplankton. The small non-diatoms were chiefly Anabaena and Scenedesmus spp. (Table 13). Thus the nannoplankton was comprised of diatoms, greens and bluegreens, with a cryptomonad present during May.

The netplanktic biomass in Saginaw Bay during 1978 was also dominanted by diatoms and greens. Bluegreens were relatively unimportant. Diatoms constituted 23.4 to 98.7% of the biomass of large cells. Stephanodiscus niagarae was overwhelmingly dominant in terms of total biomass. Tabellaria, Asterionella, and Fragillaria were of lesser importance. Large non-diatoms present were Pediastrum and Staurastrum (Table 14).

During 1979 very similar populations of phytoplankton served as test organisms for assessing the impact of PCB's. The nannoplankton were again comprised of diatoms (0.2 to 21.6%) and non-diatoms (78.4 to 99.8%). Melo-

TABLE 12. TOTAL PCB CONCENTRATION (ng $l^{-1}$) IN SPECIFIED PORTIONS OF SAGINAW BAY, 1977*

| Date | Cruise | Mean Segment 3 | Mean Station 56 |
|---|---|---|---|
| 19 April | 1 | 22 | - |
| 10 May | 2 | 7 | 7 |
| 24 May | 3 | 21 | 24 |
| 28 June | 4 | 295** | 316** |
| 19 July | 5 | 32 | 5 |
| 2 Aug. | 6 | 33 | 39 |
| 12 Sept. | 7 | 9 | 9 |
| 26 Sept. | 8 | 6 | 7 |
| 31 Oct. | 9 | 9 | 5 |

*Courtesy W. Richardson, Large Lakes Lab. U.S.-E.P.A., Grosse Ile, MI.

**High winds resuspended sediments.

TABLE 13. COMPOSITION OF NANNOPLANKTON (< 22 µm DIAMETER) DURING 1978

| Date | Depth | Relative Biomass, % and Carbon Content ($mgCm^{-3}$) | | Relative Biomass (%) of Dominants | |
|---|---|---|---|---|---|
| | | Diatoms | Non-diatoms | Diatoms | Non-diatoms |
| 6 May | 0.5 | 43.8(85.1) | 56.2(109.4) | Steph.b.(14.3) | Anabaena(23.9) |
| | 2.5 | 22.5(73.8) | 77.5(253.8) | Melosira b.(10.5) | Cryptomonas(57.5) |
| | 5.0 | 42.4(62.2) | 57.6(84.5) | Melosira b.(14.4) | Scenedesmus(26.3) |
| 10 June | 0.5 | 1.4(11.3) | 98.6(797.7) | Melosira g.(1.0) | Scenedesmus(16.9) |
| | 2.5 | 6.4(21.4) | 93.6(312.8) | Cyclo.c.(4.3) | Scenedesmus(42.5) |
| | 5.0 | 10.3(23.2) | 89.7(201.9) | Melosira g.(10.4) | Anabaena(41.0) |
| 9 July | 0.5 | 14.6(42.5) | 85.4(249.2) | Melosira g.(7.5) | Anabaena(9.2) |
| | 2.5 | 6.2(24.1) | 93.8(362.8) | " " (3.6) | Anabaena(72.6) |
| | 5.0 | 2.2(15.9) | 97.8(721.4) | " " (2.9) | Scenedesmus(23.1) |
| 13 Aug. | 0.5 | 11.2(25.2) | 88.8(199.7) | Melosira g.(10.3) | Anabaena(71.4) |
| | 2.5 | 5.6(13.0) | 94.4(219.4) | " " (5.4) | " (55.8) |
| | 5.0 | 11.6(39.3) | 88.4(298.9) | " " (8.9) | " (15.8) |
| 8 Sept. | 0.5 | 2.6(29.1) | 97.4(1105.5) | Melosira g.(2.2) | Anabaena(27.2) |
| | 2.5 | 9.5(90.3) | 90.5(855.3) | " " (7.1) | " (49.1) |
| | 5.0 | 12.5(130.1) | 87.5(910.8) | " " (7.3) | " (33.8) |

TABLE 14. COMPOSITION OF NETPLANKTON (> 22 µm DIAMETER) DURING 1978

| Date | Depth | Relative Biomass, % and Carbon Content ($mgCm^{-3}$) | | Relative Biomass (%) of Dominants | |
|---|---|---|---|---|---|
| | | Diatoms | Non-diatoms | Diatoms | Non-diatoms |
| 6 May | 0.5 | 85.8(104.8) | 14.2(17.4) | Tabellaria f.(25.0) | Pediastrum(9.7) |
| | 2.5 | 96.8(218.2) | 3.2(7.1) | Steph. n.(23.4) | " (3.2) |
| | 5.0 | 98.7(217.9) | 1.3(2.9) | " " (20.1) | - |
| 10 June | 0.5 | 62.0(181.5) | 38.0(111.3) | Steph. n.(30.4) | Pediastrum(25.1) |
| | 2.5 | 54.2(156.4) | 45.8(132.4) | Aster.(10.3) | Staurastrum(35.7) |
| | 5.0 | 39.6(125.9) | 60.4(192.4) | Fragill.(12.3) | " (50.2) |
| 9 July | 0.5 | 35.1(129.4) | 64.9(239.7) | Steph. n.(32.7) | Staurastrum(42.2) |
| | 2.5 | 47.6(195.8) | 52.4(215.6) | " " (41.1) | " (41.3) |
| | 5.0 | 23.4(172.9) | 76.6(565.7) | " " (12.2) | " (26.9) |
| 13 Aug. | 0.5 | 53.6(78.3) | 46.4(67.9) | Steph. n.(36.6) | Staurastrum(27.0) |
| | 2.5 | 41.4(66.4) | 42.4(93.8) | " " (26.3) | " (35.2) |
| | 5.0 | 58.8(74.9) | 41.2(52.4) | " " (41.6) | " (22.2) |
| 8 Sept. | 0.5 | 64.2(265.0) | 35.8(147.7) | Steph. n.(32.1) | Pediastrum(17.7) |
| | 2.5 | 41.5(213.7) | 58.5(301.3) | " " (34.9) | Staurastrum(16.5) |
| | 5.0 | 57.9(255.5) | 42.1(185.4) | " " (32.7) | " (26.9) |

sira and Cyclotella were the dominant genera of small diatoms, with the latter more abundant than during 1978 (Table 15). The small non-diatoms represented by Scenedesmus, which was dominant on all dates and all depths. Thus diatoms and greens were important, as during 1978, but bluegreens were less abundant than in 1978 (possibly due to phosphate diversion).

The netplanktic biovolume in 1978 was again represented by diatoms (0-66.0%) and non-diatoms (34.0-100%). The large diatoms were again dominated by Stephanodiscus; again Fragillaria and Asterionella were secondary. As in 1978, the large non-diatoms were Pediastrum and Staurastrum, both green algae (Table 16). We emphasize that the dichlorobiphenyl and hexachlorobiphenyl series were tested during 1978 and 1979 against very similar phytoplankton populations.

Inhibition and Stimulation of Phytoplankton Photosynthesis by Hexachlorobiphenyl and its Degradation Products

Hexachlorobiphenyl was selected as an example of a highly chlorinated isomer. As such, it was expected to be relatively toxic, since toxicity is thought to increase with chlorination. It was also suspected of being relatively refractive; slow degradation would suggest its degradation products would be difficult to find in the water, although they may occur in the livers of fishes with MFO enzymes. Lastly, penta- and hexachlorobiphenyls and their furans have been found in humans, specifically in the livers of Yusho patients, where 2, 3, 4, 7, 8 pentachlorodibenzofuran was observed (Rappe et al., 1979). Since hexachlorinated isomers are likely relatively refractive, we also decided to test a possible intermediate hydroxylated product, pentachlorobiphenylol, as well as a furan, octachlorodibenzofuran. These constituted our best approximately to a degradation series (Figure 4B). The highly chlorinated furan was selected because hexachlorodibenzofuran was unavailable.

TABLE 15. COMPOSITION OF NANNOPLANKTON (< 22 µm DIAMETER) DURING 1979

| Date | Depth | Relative Biomass, % and Carbon Content ($mgCm^{-3}$) | | Relative Biomass (%) of Dominants | |
|---|---|---|---|---|---|
| | | Diatoms | Non-diatoms | Diatoms | Non-diatoms |
| 7 June | 0.5 | 2.1(10.2) | 97.9(472.0) | Cyclotella c.(0.4) | Scenedesmus(25.3) |
| | 2.5 | 3.7(12.3) | 96.3(318.7) | " " (1.7) | " (51.7) |
| | 5.0 | 4.2(14.8) | 95.8(328.1) | " " (2.1) | " (43.8) |
| 14 July | 0.5 | 0.2(1.2) | 99.8(553.4) | Synedra n.(0.0) | Scenedesmus(24.5) |
| | 2.5 | 21.6(140.2) | 78.4(507.6) | Cyclotella c.(1.0) | " (22.6) |
| | 5.0 | 3.3(25.9) | 96.7(726.3) | " " (5.4) | " (15.6) |
| 16 Aug. | 0.5 | 1.9(29.8) | 98.1(1533.8) | Cyclotella c.(0.8) | Scenedesmus(16.8) |
| | 2.5 | 3.6(58.4) | 96.4(1577.2) | Melosira(2.2) | " (14.6) |
| | 5.0 | 2.4(24.5) | 97.6(978.1) | Cyclotella(0.9) | " (51.7) |
| 18 Sept. | 0.5 | 5.7(120.6) | 94.3(2000.9) | Melosira(5.2) | Scenedesmus(15.3) |
| | 2.5 | 6.5(132.6) | 93.5(1912.8) | " (5.6) | " (11.5) |
| | 5.0 | 9.1(139.9) | 90.1(1398.1) | " (5.1) | " (11.4) |

TABLE16. COMPOSITION OF NETPLANKTON (> 22 µm DIAMETER) DURING 1979

| Date | Depth | Relative Biomass, % and Carbon Content ($mgCm^{-3}$) | | Relative Biomass (%) of Dominants | |
|---|---|---|---|---|---|
| | | Diatoms | Non-diatoms | Diatoms | Non-diatoms |
| 7 June | 0.5 | 15.1(22.3) | 84.9(125.9) | Stephanodiscus(5.2) | Staurastrum(57.4) |
| | 2.5 | 35.4(37.7) | 64.6(68.9) | " (27.3) | " (39.9) |
| | 5.0 | 22.3(72.9) | 77.7(254.4) | " (5.7) | Pediastrum(29.8) |
| 14 July | 0.5 | 0.0(0) | 100.0(267.2) | - | Staurastrum(69.4) |
| | 2.5 | 2.2(8.4) | 97.8(373.1) | Rhizoselenia(0.7) | " (19.4) |
| | 5.0 | 3.6(5.2) | 96.4(140.2) | Fragillaria c.(7.2) | Pediastrum(46.1) |
| 16 Aug. | 0.5 | 4.1(16.2) | 95.9(375.2) | Asterionella(2.3) | Pediastrum(46.2) |
| | 2.5 | 15.7(45.1) | 84.3(241.5) | " (7.0) | Staurastrum(39.6) |
| | 5.0 | 11.2(74.7) | 88.8(589.7) | Stephanodiscus(6.3) | Pediastrum(29.1) |
| 18 Sept. | 0.5 | 66.0(137.7) | 34.0(71.0) | " (42.2) | " (27.8) |
| | 2.5 | 57.5(260.6) | 42.5(192.9) | " (34.8) | Staurastrum(18.7) |
| | 5.0 | 56.5(230.4) | 43.4(176.7) | " (41.8) | " (24.4) |

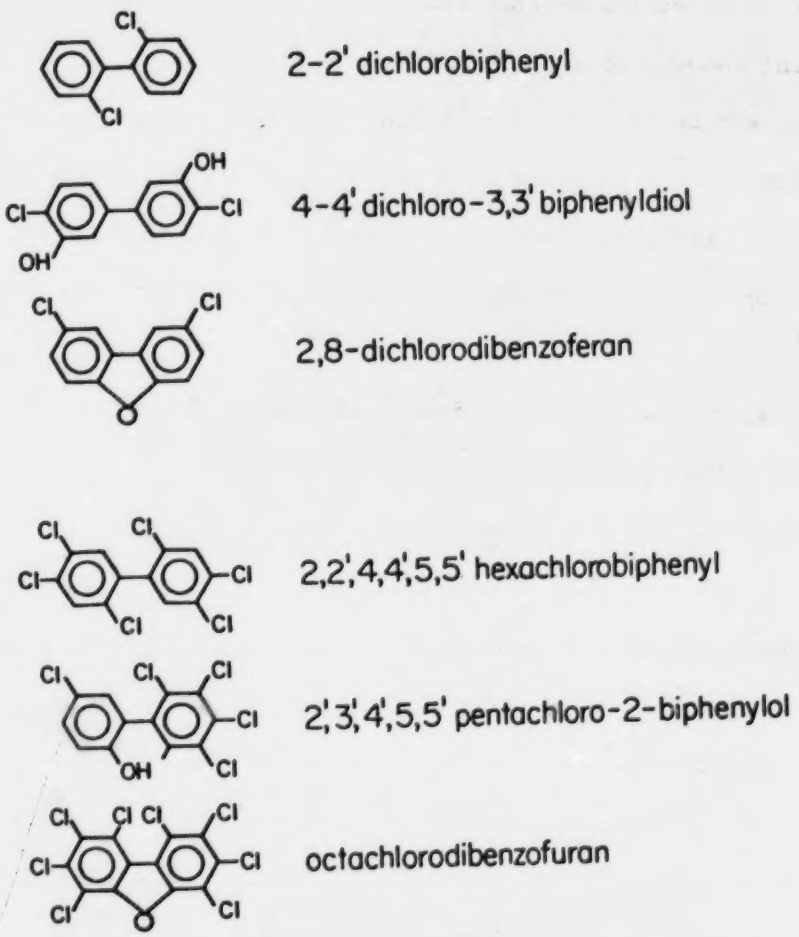


Figure 4A. Simulated route of transfer of dichlorobiphenyl to dichlorodibenzofuran (di- series).

Figure 4B. Simulated route of transfer of hexachlorobiphenyl to its furan (hexa- series).

Detailed Monthly Results--

The mean monthly inhibition (stimulation by 100 ng $l^{-1}$ PCB) of carbon fixation by nannoplankton and netplankton, relative to the controls, is shown in Table 17. First we will discuss the results from surface samples from two months. Then we will summarize effects of PCB's with depth. The objective of examining results of surface incubation and resulting carbon fixation was to note the effect of increasing exposure to PCB's. Examining results with depth enabled us to observe the effect of light intensity on inhibition/stimulation of photosynthesis in the presence of PCB's.

The results of the experiment of 10 June 1978 represent an example where phytoplankton photosynthesis was relatively equivalent at the three depths (Table17); also, there was no evidence of increased surface inhibition due to the effect of increased exposure to PCB's (Figure 5 ). Nannoplankton (o) were inhibited by 5 ng $l^{-1}$ to the same extent as by 500 ng $l^{-1}$. The hydroxylated PCB (OH-PCB) and the furan (CDBF-PCB) caused slightly more inhibition. Netplankton (•) likewise were not effected much by an increase in exposure from 5 ng $l^{-1}$ to 500 ng $l^{-1}$. The OH-PCB was slightly more inhibitory and the CDBF-PCB slightly less inhibitory, as compared to the parent isomer (Figure 5). We suspect that this example was representative of inhibition in a well mixed water column; the wind velocity at noon was 12-24 mph from the SW. Returning to Table , we see that the nannoplankton population was inhibited at 5 m. The particular phytoplankton asemblage involved was dominated (biovolume) by a small diatom, Melosira (17.6%) and a large green, Pediastrum (41%).

The experiment of 9 July 1978 portrays strong surface inhibition with increasing exposure to hexachloro ((5-500 ng $l^{-1}$); as illustrated in Figure 6.

TABLE 17. RELATIVE EFFECTS OF 100 ng $l^{-1}$ HEXACHLOROBIPHENYL SERIES UPON NANNOPLANKTON AND NETPLANKTON PHOTOSYNTHESIS (% INHIBITION/STIMULATION)

| Date and Depth | Nannoplankton Treatment | | | Netplankton Treatment | | |
|---|---|---|---|---|---|---|
| | Parent Isomer | OH-PCB | CDBF | Parent Isomer | OH-PCB | CDBF |
| 6 May 1978 | | | | | | |
| 0.5 m | -37.7 | -31.9 | -21.5 | -42.8 | -21.4 | -22.1 |
| 2.5 m | -31.9 | +13.2 | 0 | -18.4 | -10.7 | +5.3 |
| 5.0 m | +5.3 | -5.4 | -8.9 | +56.9 | +46.6 | +18.1 |
| 10 June 1978 | | | | | | |
| 0.5 | -22.3 | -37.4 | -31.3 | -22.0 | -25.7 | -17.6 |
| 2.5 | - | - | - | -8.1 | +8.5 | -7.0 |
| 5.0 | -17.4 | -23.8 | -38.3 | -5.6 | +46.3 | +48.1 |
| 9 July 1978 | | | | | | |
| 0.5 | -46.6 | -74.1 | - | -5.8 | -93.6 | - |
| 2.5 | -25.3 | -49.4 | +0.3 | -13.4 | -2.1 | -8.0 |
| 5.0 | +156.0 | +162.0 | +106.0 | +34.9 | +30.2 | +53.3 |
| 13 August 1978 | | | | | | |
| 0.5 | +1.9 | -16.0 | +12.7 | -18.3 | -10.0 | -41.1 |
| 2.5 | +12.0 | -10.7 | -3.6 | -4.3 | -19.0 | -10.6 |
| 5.0 | +4.6 | -9.2 | +53.1 | +7.0 | -4.9 | -7.8 |
| 8 September 1978 | | | | | | |
| 0.5 | +4.5 | -7.0 | +1.0 | -18.1 | -20.7 | -22.7 |
| 2.5 | +3.9 | +3.9 | +18.1 | +25.3 | +25.6 | +7.0 |
| 5.0 | -44.1 | -27.0 | -22.3 | +9.6 | -10.0 | -11.4 |

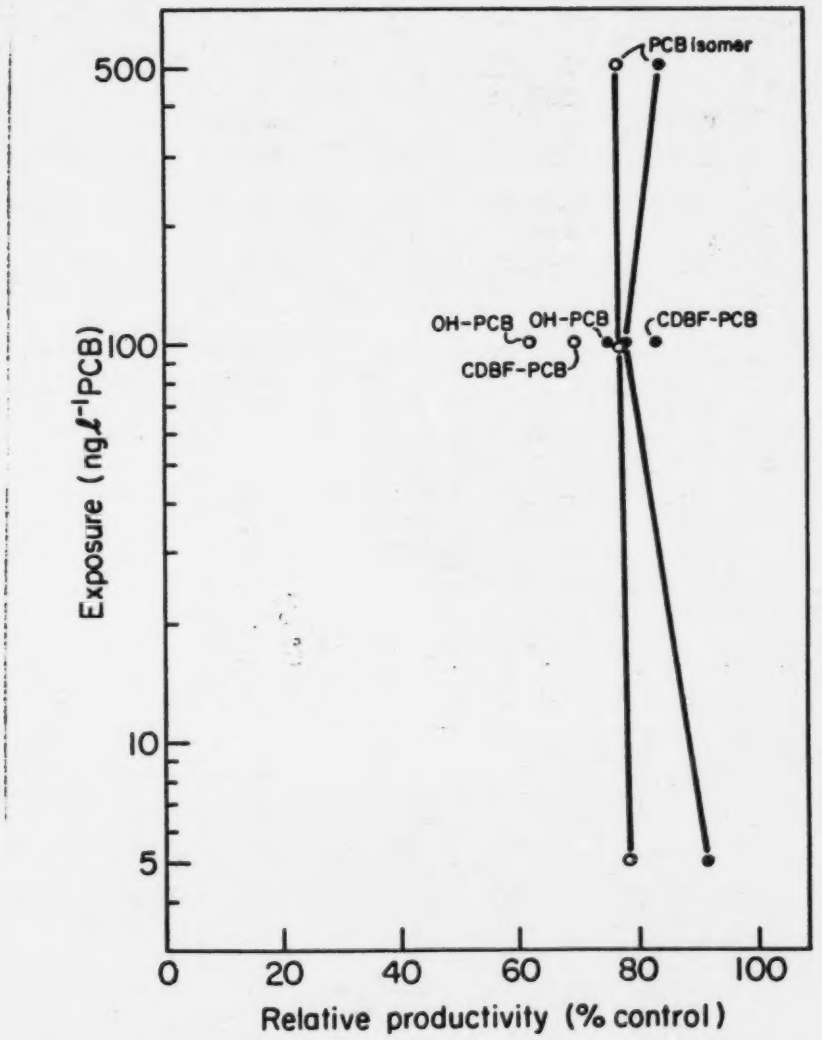


Figure 5. Relative nannoplankton (o) and netplankton (o) productivity on 10 June 1978 at 0.5 m depth upon exposure to 5, 100 and 500 ng $l^{-1}$ hexachlorobiphenyl and 100 ng $l^{-1}$ pentachlorobiphenylol (OH-PCB) and octachlorodibenzofuran (CDBF).

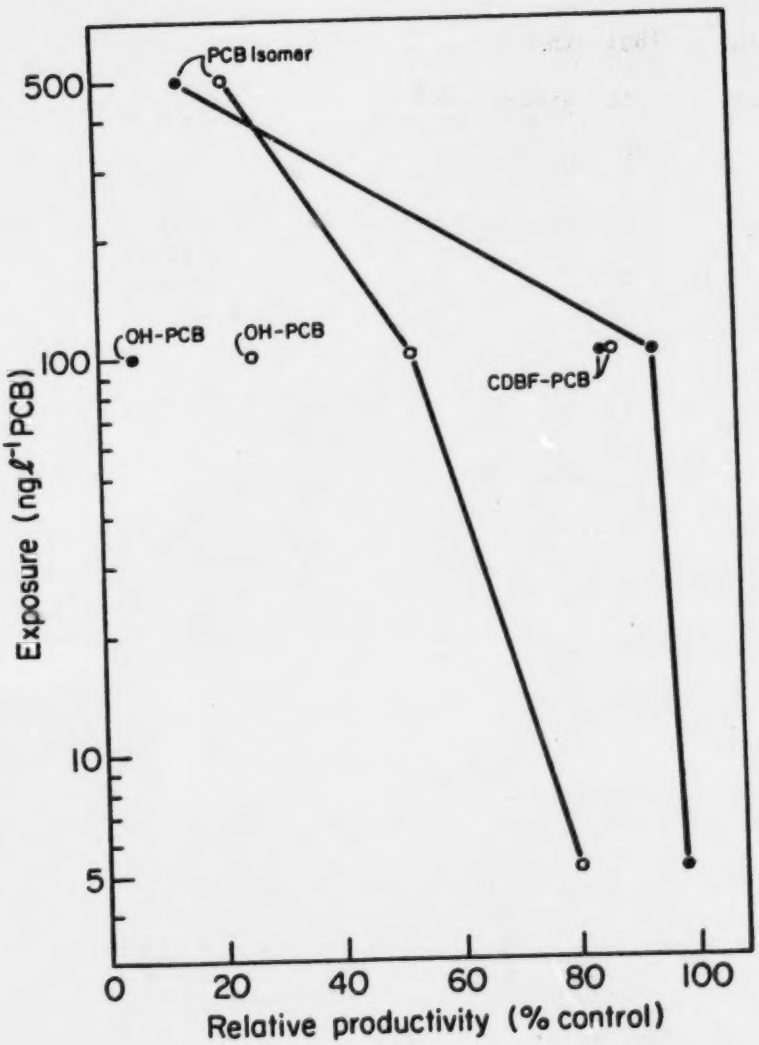


Figure 6. Relative nannoplankton (o) and netplankton (o) productivity on 9 July 1978 when exposed to hexa-series at 0.5 m depth.

Relative productivity dropped with increased exposure for both nannoplankton (o) and netplankton (●). The nannoplankton was dominated by Scenedesmus (21.7%), where as the netplankton biomass was chiefly Stephanodiscus niagarae (32.7%). The CDBF-PCB was less or about equally inhibitive to photosynthesis as the parent isomer. The hydroxylated product was extremely inhibitive; the nannoplankton were inhibited 74.1% (or productivity was 25.9% of control). Whereas the large diatom Stephanodiscus and the rest of the netplankton was inhibited 93.6%. Again the diatoms are often inhibited the most. Again we suspected previous that this example was representative of inhibition during a calm speel of weather; the wind velocity during the experiment varied from calm to 7 mph from the SW.

Estimate of Seasonal Inhibition of Nannoplankton by Hexachlorobiphenyl in Saginaw Bay--

Total PCB concentrations in Saginaw Bay vary between 5 and 316 ng $l^{-1}$. It is very difficult to estimate how much is soluble or "available" PCB; however, Eisenreich (1980) has demonstrated for L. Superior that more is soluble than particulate. At Station 56 we measured nannoplankton productivity ($mgCm^{-2}$ $hr^{-1}$) for the water column (Figure 7). From experimentally measured inhibition due to PCB's at concentrations of 5, 100 and 500 ng $l^{-1}$, we should be able to make interpolative estimates for inhibition over the natural range of 5-316 ng $l^{-1}$. Clearly such inhibition is usually beloe 10%; however, following storms which resuspend the PCB rich sediments, waterborne PCB's may reach 316 ng $l^{-1}$ and inhibit productivity more than 30% (Figure 7). Thus significant PCB effects upon algae may be linked to turbulence.

Estimates of Threshold Effects from Low Concentrations of PCB's--

Intensive studies utilizing exposure of natural populations of nannoplankton and netplankton to 5 ng $l^{-1}$ hexachlorobiphenyl enable us to examine

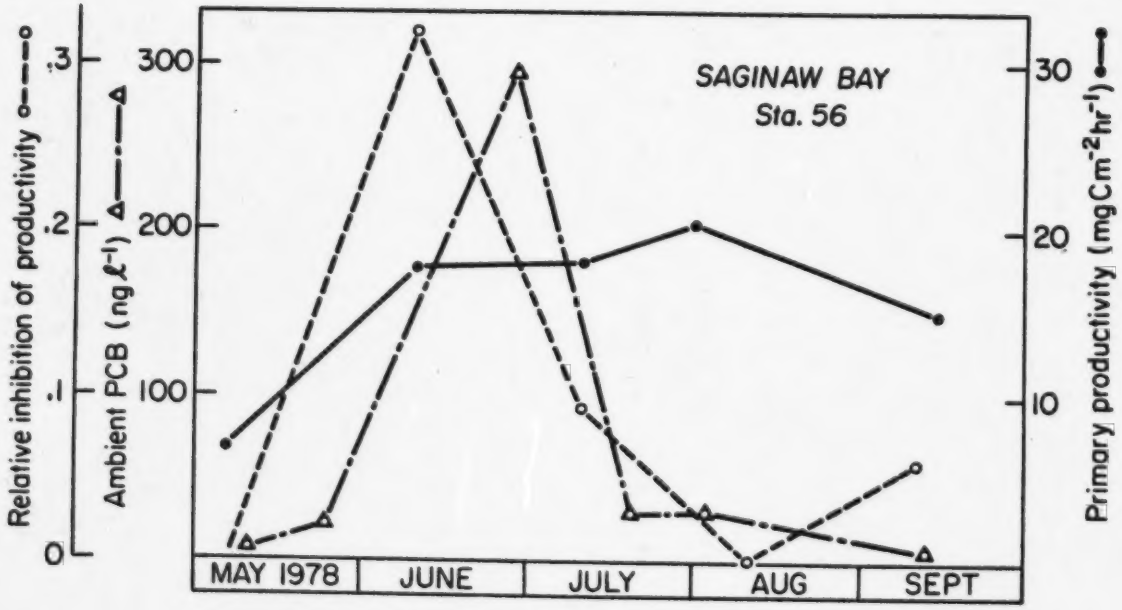


Figure 7. Estimated relative inhibition of productivity (o) relative to actual productivity (o) and PCB levels (ng $l^{-1}$).

thresholds for inhibition. On three occasions (6 May, 10 June and 9 July) (Table 18), nannoplanktic populations algal dominated by Melosira and Scenedesmus, showed inhibitions greater than 20%. Thus we feel we have established a new lower threshold for the effects of PCB's on carbon fixation of 5 ng $l^{-1}$ (utilizing a 4 hr incubation).

Dark uptake of carbon is stimulated by PCB's. Now we can suggest a threshold for dark stimulation of 5 ng $l^{-1}$. On four of five occasions populations dominated by Scenedesmus, Scenedesmus, Anabaena and Anabaena were stimulated 21 to 80% with the addition of 5 ng $l^{-1}$ hexachlorobiphenyl.

These threshold levels of 5 ng $l^{-1}$ are in order of magnitude lower than previously reported. Harding (1976), Powers et al. (1977), Biggs et al. (1978), and Harding and Phillips (1978) all found thresholds of 10 µg $l^{-1}$ for suppression of photosynthesis. Moore and Harriss (197 ) found a threshold of 7 µg $l^{-1}$ for inhibition by 2,4'dichlorobiphenyl.

Inhibition and Stimulation of Phytoplankton Photosynthesis by Dichlorobiphenyl and its Degradation Products

Dichlorobiphenyl was selected as an example of a relatively unchlorinated isomer. It was expected to be relatively less toxic than hexachlorobiphenyl and more rapidly degraded. Degradation models have suggested formation of the hydroxylated intermediate in lakes within 2 years (Bunce et al., 1978). It hs not been found in humans to the degree that the penta- and hexachlorobiphenyls have.

Detailed Monthly Result--

Dichlorobiphenyl was not particularly inhibitory to the phytoplankton on 16 August 1979. In surface waters the nannoplankton (o) and netplankton (o) showed little response to increased exposure. The nannoplankton inhibited were 12.7% at 5 ng $l^{-1}$ and stimulated 4.4% at 500 ng $l^{-1}$. The nannoplankton,

TABLE 18. THRESHOLD EFFECTS OF 5 ng $l^{-1}$ HEXACHLOROBIPHENYL SERIES UPON NANNOPLANKTON AND NETPLANKTON PHOTOSYNTHESIS (% INHIBITION/STIMULATION

| Date and Depth | Nannoplankton | Netplankton |
|---|---|---|
| | Treatment = Parent Isomer | Treatment = Parent Isomer |
| 6 May 1978 | | |
| 0.5 | -20.7 | - |
| 2.5 | +28.3 | -12.3 |
| 5.0 | +6.5 | +68.1 |
| 10 June 1978 | | |
| 0.5 | -22.3 | -8.7 |
| 2.5 | - | -6.3 |
| 5.0 | -8.5 | +80.2 |
| 9 July 1978 | | |
| 0.5 | -20.1 | -1.4 |
| 2.5 | -5.7 | -11.7 |
| 5.0 | +105.0 | +21.7 |
| 13 August 1978 | | |
| 0.5 | -2.0 | -17.4 |
| 2.5 | +10.4 | -14.7 |
| 5.0 | -10.9 | +29.7 |
| 8 Sept. 1978 | | |
| 0.5 | -13.5 | -3.3 |
| 2.5 | +18.7 | +7.3 |
| 5.0 | -33.5 | -11.0 |

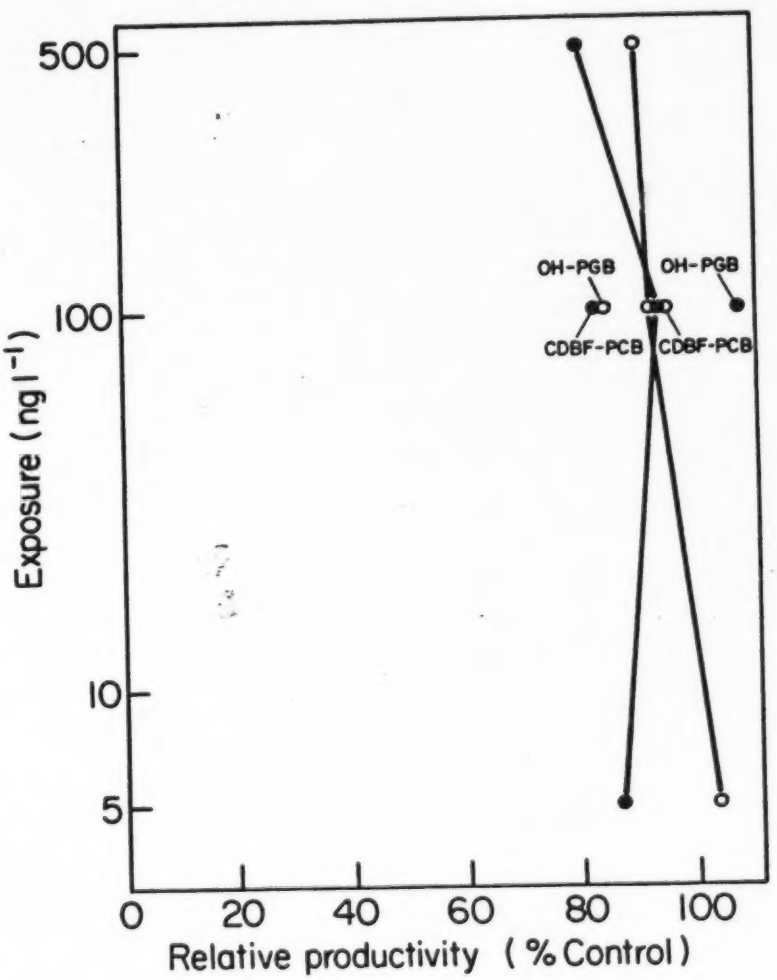


Figure 8. Relative nannoplankton (o) and netplankton (o) productivity at 0.5 m depth on 16 August 1979 when exposed to dichlorobiphenyl series.

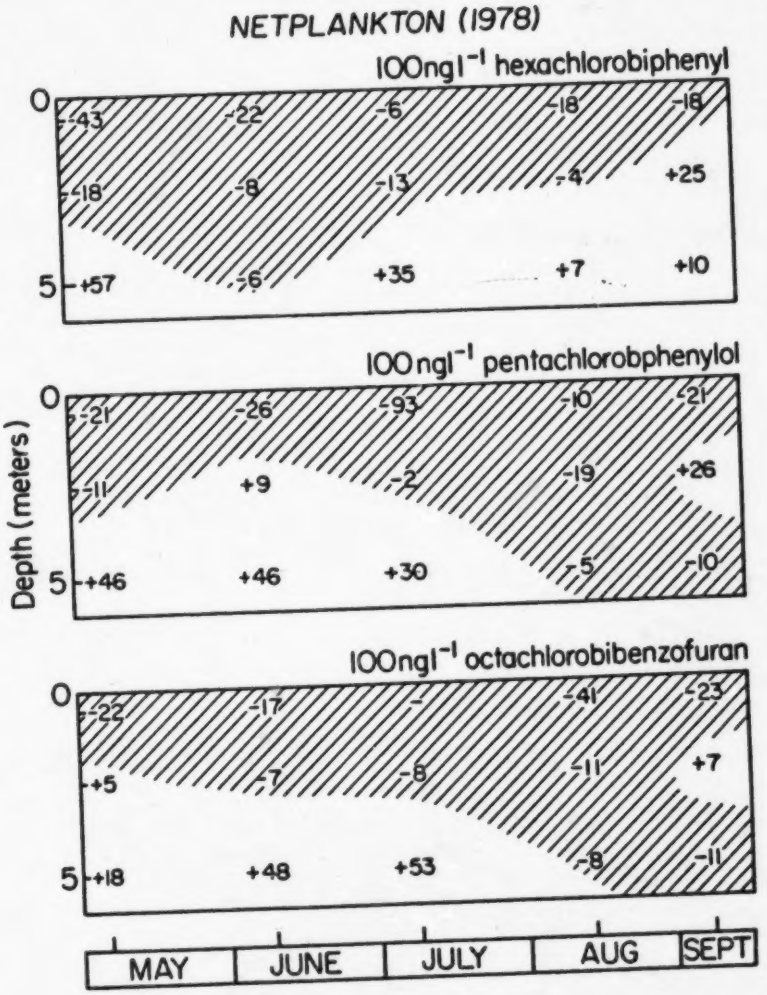


Figure 9. Inhibition (hatched) and stimulation of netplankton carbon fixation by hexa- series (100 ng $l^{-1}$) during 1978.

dominated by Cyclotella, were inhibited by the CDFB (16.2%) and only slightly less by the hydroxylated product (7.0%). The netplankton (●), dominated by Scenedesmus, likewise showed little inhibition at any exposure (Figure 8). The furan inhibited photosynthesis 7.4%. Generally we may conclude that 2,2' PCB had little effect on carbon fixation at or above ambient concentrations (39 ng $l^{-1}$).

Seasonal Summary - 2,2' PCB Series--

The effects of 2,2' PCB's on the phytoplankton are basically to inhibit photosynthesis at the surface and stimulate carbon uptake at depth. The nannoplankton were inhibited at all depths by the parent isomer during June 1979 (Figure 8). By August, photosynthesis was stimulated at all depths, with carbon uptake at 5 m about two times the control. Remember that phytoplankton populations were dominated in the spring by the small green algae, Scenedesmus, which carried into August. The hydroxylated product also inhibited photosynthesis during the spring, whereas the CDBF was inhibitory to nannoplankton at 5 m in spring and fall and stimulatory at depth in July and August.

From Table 19 we have calculated that the mean stimulation to nannoplankton photosynthesis of dichlorobiphenyl (100 ng $l^{-1}$) over the column was 31.1%, while that of the hydroxylated product was 10.0%, while inhibition due to the CDBF was 1.7%. Thus the effects of dark stimulation of carbon uptake by nannoplankton resulted in a net column stimulation for the parent isomer and the hydroxylated product. Only the furan was slightly inhibitory.

The netplankton, chiefly Stephanodiscus and Staurastrum, were likewise frequently inhibited in surface light by 100 ng $l^{-1}$ of the three PCB compounds. Of the surface samples, 11 of 12 showed inhibition (Table 19). How-

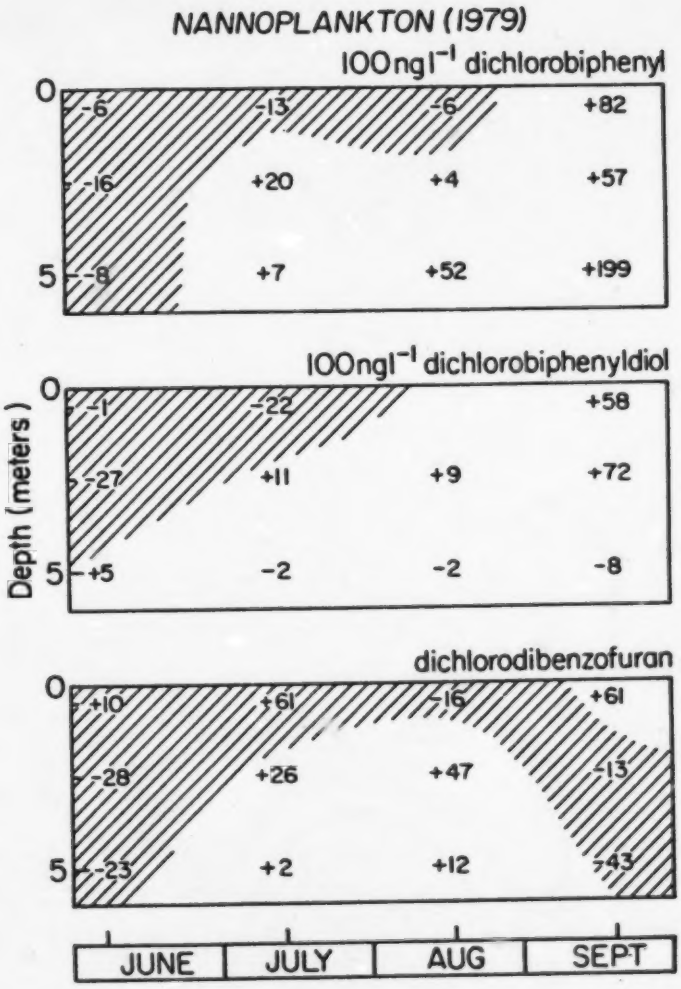


Figure 10. Inhibition (hatched) and stimulation of nannoplankton carbon fixation by di- series (100 ng $l^{-1}$) during 1979.

TABLE 19. RELATIVE EFFECTS OF 100 ng $l^{-1}$ DICHLOROBIPHENYL UPON NANNOPLANKTON AND NETPLANKTON PHOTOSYNTHESIS (% INHIBITION/STIMULATION)

| Date and Depth | Nannoplankton Treatment | | | Netplankton Treatment | | |
|---|---|---|---|---|---|---|
| | Parent Isomer | OH-PCB | CDBF | Parent Isomer | OH-PCB | CDBF |
| 7 June 1979 | | | | | | |
| 0.5 m | -6.15 | -0.95 | +9.58 | -9.11 | -18.13 | -19.30 |
| 2.5 m | -15.64 | -27.27 | -22.25 | -12.53 | -16.40 | +1.7 |
| 5.0 m | -7.56 | +4.84 | -22.57 | +6.23 | -28.36 | +3.11 |
| 14 July 1979 | | | | | | |
| 0.5 | -13.31 | -22.04 | -61.5 | -1.92 | -21.04 | +91.5 |
| 2.5 | +20.32 | +11.82 | +26.78 | -1.79 | -5.98 | +1.7 |
| 5.0 | +6.73 | -1.90 | +2.08 | +65.3 | +22.81 | +11.08 |
| 16 Aug. 1979 | | | | | | |
| 0.5 | -5.7 | - | -16.2 | -7.20 | -15.87.4 | |
| 2.5 | +4.7 | +9.0 | +47.2 | +5.4 | +15.0 | +27.9 |
| 5.0 | +52.7 | -1.7 | +11.8 | -15.6 | +11.5 | +0.3 |
| Sept. 1979 | | | | | | |
| 0.5 | +81.5 | +58.4 | +61.2 | -78.8 | -50.8 | -48.7 |
| 2.5 | +57.3 | +71.7 | -13.9 | -2.5 | +28.4 | +104.9 |
| 5.0 | +199.2 | -8.3 | -43.3 | +54.7 | +64.9 | +110.2 |

ever, the mean stimulation of netplankton in the water column was 0.1% for the parent isomer, an inhibition of 1.1% for the hydroxylated product, and stimulation of 16.1% for the CDBF. Again the effect of dark stimulation by PCB of carbon uptake was apparent. These striking results stress the importance of working at typical ambient levels of PCB's for Saginaw Bay (Table 19).

Thus PCB's must be considered as both stimulatory and inhibitory to algal photosynthesis. Those factors effecting dark stimulation and light inhibition must be clearly understood.

Factors Interacting to Reduce Carbon Fixation

Relative Chlorination of PCB's Versus Light Intensity--

The literature suggests that toxicity of PCB's increases with chlorination. In turn, the light intensity at the surface in Saginaw Bay is often 100 X that at 5 m depth. We see that the three isomers of the hexa- series inhibited both netplankton and nannoplankton in surface waters (Figure 9). In contrast only two isomers, the present 2,2' and the hydroxylated product, inhibited the netplankton; in contrast, the nannoplankton were stimulated by all three isomers of the 2,2' series (Fig. 10) over the entire water colume the 2,2' series was predominantly stimulatory to carbon fixation, while the hexa- series was inhibitory (Figure11), illustrating the effects of chlorination

Threshold for Dark Stimulation of Carbon Fixation in Presence PCB--

Since absence of light is such an important factor in understanding the effects of PCB's upon photosynthesis, and since individual species act differently, we have plotted the inhibition/stimulation of hexachlorobiphenyl and its hydroxylated product against light intensity. For both compounds, stimulation of carbon fixation in populations dominated by the diatom Melosira occurs between 2 and 10% of maximum daily light intensity (Figure 12). Such intensities are found at depths of 2.5-5 m during the summertime in

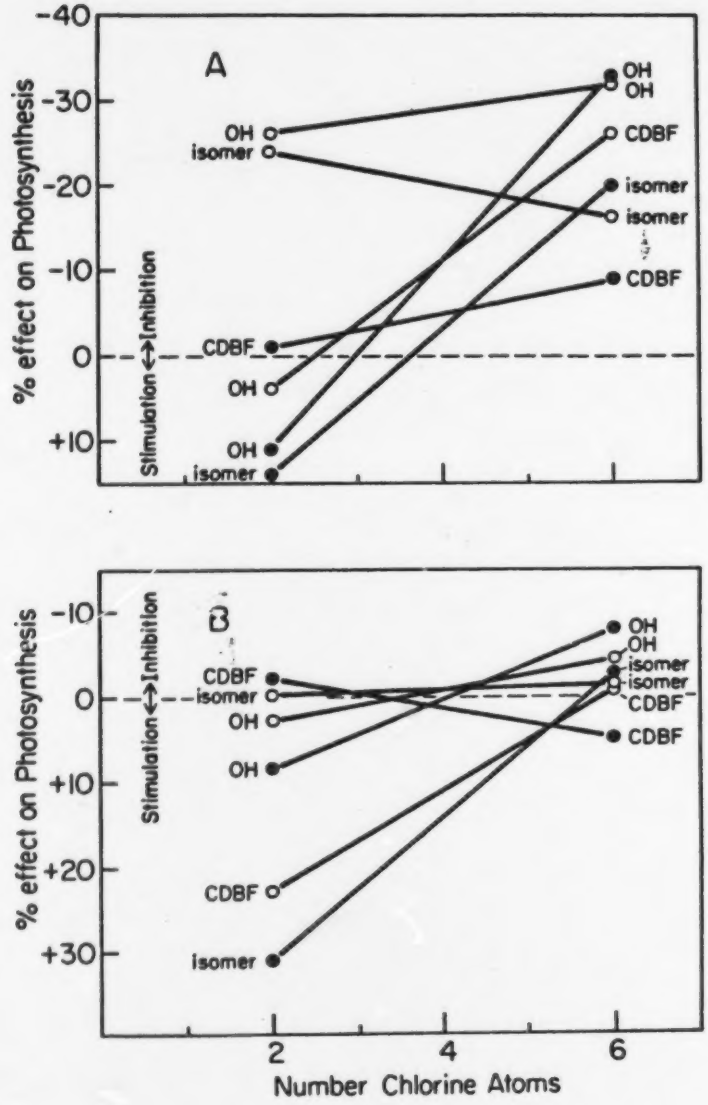


Figure 11. Stimulation/inhibition of carbon fixation by PCB's and metabolites relative to degree of chlorination.

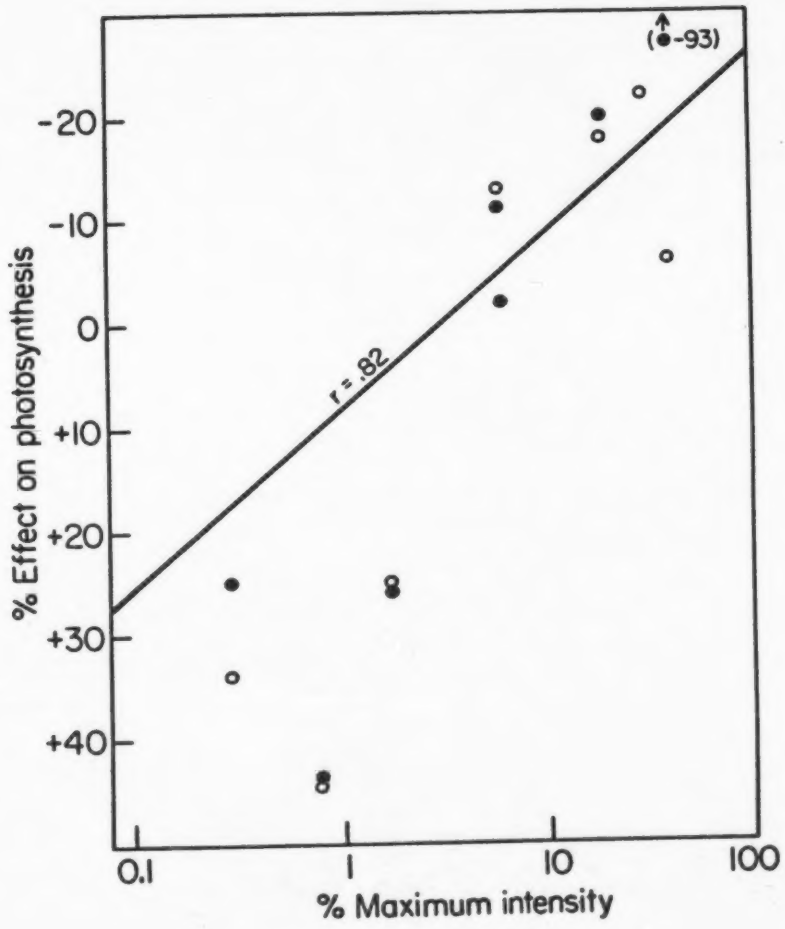


Figure 12. Stimulation/inhibition of carbon fixation by 100 ng $l^{-1}$ hexachlorobiphenyl (o) and its hydroxylated product (o) relative to light intensity.

Saginaw Bay. On the other hand, inhibition of carbon fixation above 15% occurs above 15% of maximum light available to the organisms.

Partition Coefficients and Inhibition--

The lipid-water partition coefficients (log P) for the di- and hexa-series of isomers should predict their effect upon photosynthesis. However, partition coefficients for PCB's are hard to obtain from the literature. Veith (1979) found coefficients ranging from 6 to 7. Pavlou and Dexter (1979) measured the water-lipid coefficient for zooplankton for hexachlorobiphenyl (Table 20). Leo et al. (1971) provided a model for the determination of the effects of hydroxyls and furan groups on partition coefficients. We have calculated partition coefficients for each of the six compounds (Table 20). Generally chemicals with a log P of 4 and greater cannot be measured with precision (Veith, 1979).

The partition coefficients of the parent isomers (di- and hexa-) and CDBF overlap; these isomers are generally less inhibitory than their corresponding hydroxylated products. For the parent isomers inhibition of carbon fixation (acute toxicity) increased with log P. The same was true for the CDBF; the octa-DBF had a larger log P and was more inhibitory.

The hydroxylated products were characterized by low estimated log P values (Table ), but within the OH effects toxicity increased as log P. Note that the toxicity of the hydroxylated products to netplankton (o) and nannoplankton (o) were the highest of the three products for any value of log P (Figure 13).

TABLE 20. PARTITION COEFFICIENTS FOR DI- AND HEXACHLOROBIPHENYL ISOMERS AND THEIR SUSPECTED DEGRADATION PRODUCTS, BOTH MEASURED AND CALCULATED

| Isomer and Degradation Products | Partition Coefficient (P), lipid-water | Correction for hydroxyl and furan groups | log P |
|---|---|---|---|
| **Dichlorobiphenyl Series** | | | |
| 2,2' dichlorobiphenyl | $0.4 \times 10^4$ | | 3.60 |
| 4,4' dichloro- 3,3' biphenyldiol | | -2(1.16)** | 1.28 |
| 2,8 dichlorodibenzofuran | | -.98** | 2.62 |
| **Hexachlorobiphenyl Series** | | | |
| 2, 2', 4, 4', 5, 5' hexachlorobiphenyl | $2.41 \times 10^{6*}$ | | 6.38 |
| 2', 3', 4', 5, 5' pentachloro -2 biphenylol | | -1.16** | 5.22 |
| octachlorodibenzofuran | | -.98** | 5.40 |

* from Pavlov and Dexter, 1980.
** from Leo, Hanch and Elkins, 1971.

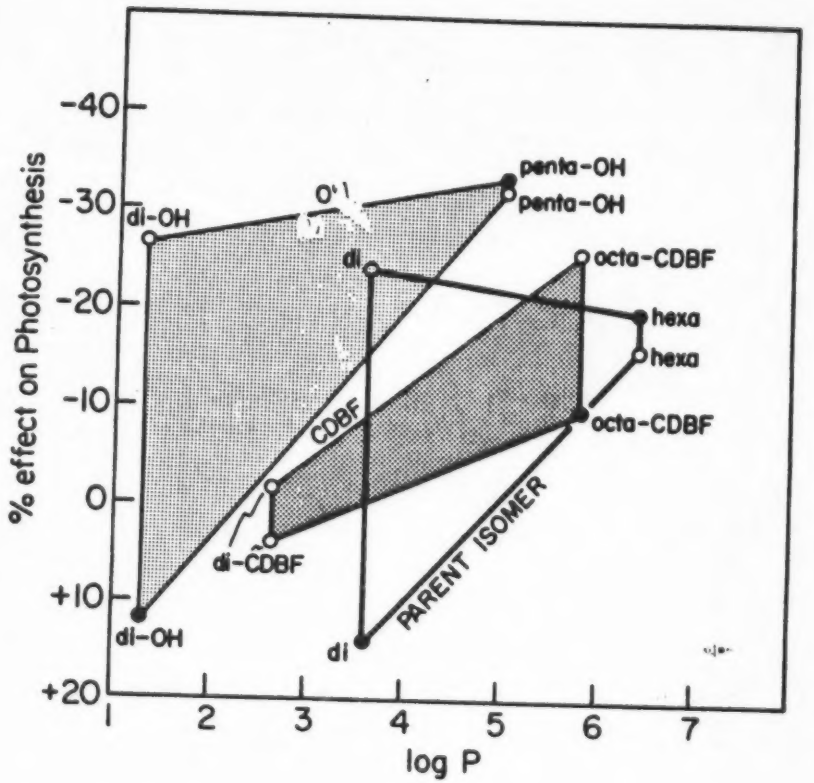


Figure 13. Stimulation/inhibition of carbon fixation by di- and hexachlorobiphenyl and metabolites relative to their lipid-water partition coefficients.

## 5.3 CHRONIC EFFECTS OF DICHLOROBIPHENYL UPON THE SURVIVAL OF THE CRUSTACEAN DIAPTOMUS

### Purpose

The chronic effects of dichlorobiphenyl were examined by exposing the zooplankter Diaptomus to three concentrations of PCB's, with control organisms held in lake water. The presence of food was also regulated.

### Results

Results of May 1978: Effects of PCB concentration upon survival of Diaptomus.

The experimental apparatus, including flasks with animals, pumps and PCB stock solutions were placed in a constant temperature room at 11.1°C.

The Diaptomus all died after 14 days (Table 21). The animals in flasks receiving 100 ng $l^{-1}$ showed reduced movement, and often it was difficult to tell whether they were living, even under a dissecting microscope. If necessary, they were touched with a probe to elicit movement. Similar observations have been made on zooplankton subjected to thermal shock. This demonstration of induced torpor shows the locomotory effects of minimal, environmentally realistic concentrations of PCB upon Diaptomus.

Concentrations ranging from 100 ng $l^{-1}$ to 10 µg $l^{-1}$ cannot be shown to have major effects upon Diaptomus, certainly not proportionally to exposure (Table ). The number of animals surviving in 100 ng $l^{-1}$ PCB after 14 days (8.2 $\pm$ 1.3) and in 1 µg $l^{-1}$ PCB (6.8 $\pm$ 2.0) was not significantly less than that surviving in the control (6.8 $\pm$ 1.3). However, significantly fewer animals (2.4 $\pm$ 1.8) survived in 10 µg $l^{-1}$ PCB. Thus the effect of concentration was only observed at a relatively high level of exposure (10 µg $l^{-1}$) not found in the natural environments of Lake Huron, and not even in the relatively contaminated waters of Saginaw Bay.

TABLE 21. NUMBER OF SURVIVING, STARVED *DIAPTOMUS* IN FLASKS EXPOSED TO THREE CONCENTRATIONS (100 ng $l^{-1}$, 1 µg $l^{-1}$, 10 µg $l^{-1}$) OF 2,2' DICHLOROBIPHENYL, BEGINNING MAY 18, 1978

| Day | Control | 100 ng $l^{-1}$ | 1 µg $l^{-1}$ | 10 µg $l^{-1}$ |
|---|---|---|---|---|
| 0 | 10.0 | 10.0 | 10.0 | 10.0 |
| 1 | 9.2 ± .83 | 10.0 | 9.0 ± 1.4 | 9.0 ± 1.4 |
| 4 | 8.6 ± .56 | 9.6 ± 1.5 | 8.8 ± 1.0 | 6.1 ± 1.6 |
| 5 | 8.3 ± .48 | 9.0 ± 1.1 | 8.8 ± 1.1 | 5.1 ± 1.9 |
| 6 | 7.8 ± .63 | 9.2 ± 1.2 | 8.2 ± 1.2 | 5.6 ± 2.2 |
| 7 | 7.6 ± .54 | 8.4 ± 2.0 | | |
| 8 | 7.4 ± .54 | 8.4 ± 2.0 | 8.6 ± 1.3 | 5.8 ± 1.9 |
| 12 | 5.2 ± 1.7 | 8.6 ± 1.2 | 6.3 ± 1.9 | 2.6 ± 1.2 |
| 13 | 7.0 ± 0 | 8.0 ± 1.4 | 7.6 ± 2.5 | 2.8 ± 1.3 |
| 14 | 6.8 ± 1.3 | 8.2 ± 1.3 | 6.8 ± 2.0 | 2.4 ± 1.8 |

Results of 12 March 1979: Effects of PCB concentration upon survival.

The initial experiment examining the effects of exposure level upon the survival of Diaptomus was repeated with similar results to the previous experiment (Table 22). Animals exposed to 3.43 µg $l^{-1}$ PCB and 20 µg $l^{-1}$ PCB survived longer than animals grown in lake water (controls). At 21 days survival was significantly longer in 3.43 and 20 µg $l^{-1}$ PCB than in lakewater alone. Clearly we were unable to demonstrate long-term chronic mortality of Diaptomus through exposure in flow-through chambers to ambient levels of PCB's characteristic or exceeding those of the Great Lakes.

Results of 10 April 1979: Effects on survival of PCB's combined with food.

One hypothesis suggested that equilibrium partitioning of PCB's at low concentrations might not result in zooplankton mortality. However, if nannoplankton foods (1000 cells $l^{-1}$ Chlamydomonas) were added to the stock bottles previous to the addition of dichlorobiphenyl, we might expect to involve another step in the physical-biological concentration of PCB's, leading to greater bioconcentration higher in the food chain, and a significant mortality of a herbivore like Diaptomus.

The addition of food to adsorb PCB's did not increase the mortality of Diaptomus at high PCB concentrations. The numbers of Diaptomus living after 30 days at 3.43 µg $l^{-1}$ and 20 µg $l^{-1}$, as well as in the controls (Table 23), were not significantly different. Clearly food was not the critical interacting factor which we sought. The addition of small Chlamydomonas did not significantly increase the mortality of Diaptomus in experimental treatments involving PCB's. In fact, a larger but insignficant number of animals survived in the presence of dichlorobiphenyl.

TABLE 22. NUMBER OF SURVIVING, STARVED *DIAPTOMUS* (MALES) IN FLASKS EXPOSED TO CONCENTRATIONS OF 3.43 µg $l^{-1}$ AND 20 µg $l^{-1}$) OF 2,2' DICHLOROBIPHENYL, BEGINNING 12 MARCH, 1979

| Day | Control | 3.43 µg $l^{-1}$ | 20 µg $l^{-1}$ |
|---|---|---|---|
| 0 | 10 | 10 | 10 |
| 1 | 9.8 | 9.8 | 9.2 |
| 2 | 9.8 | 9.8 | 9.0 |
| 3 | 9.6 | 9.5 | 8.8 |
| 4 | 9.2 | 9.5 | 8.6 |
| 7 | 8.8 | 8.5 | 8.0 |
| 8 | 8.0 | 8.5 | 7.8 |
| 9 | 7.6 | 8.0 | 7.6 |
| 10 | 7.2 | 8.0 | 7.2 |
| 11 | 6.0 | 7.5 | 6.4 |
| 14 | 4.4 | 7.0 | 6.4 |
| 15 | 4.0 | 6.5 | 6.4 |
| 16 | 3.4 | 5.8 | 6.4 |
| 17 | 3.0 | 5.3 | 6.0 |
| 18 | 3.0 | 5.0 | 6.0 |
| 21 | 2.4 | 4.3 | 5.2 |

TABLE 23. NUMBER OF SURVIVING, WELL FED *DIAPTOMUS* (MALES) IN FLASKS EXPOSED CONCENTRATIONS (3.43 µg $l^{-1}$ and 20 µg $l^{-1}$) OF 2,2' DICHLOROBIPHENYL, BEGINNING 10 APRIL, 1979. ANIMALS FED 1000 CELLS $l^{-1}$ CHLAMYDOMONAS

| Day | Control | 3.43 µg $l^{-1}$ | 20 µg $l^{-1}$ |
|---|---|---|---|
| 0 | 10.0 | 9.0 | 10.6 |
| 1 | 9.8 ± .44 | 7.2 ± 4.2 | 10.4 ± 1.5 |
| 2 | 9.6 ± .54 | 8.6 ± 2.0 | 10.2 ± 1.6 |
| 3 | 9.6 ± .54 | 8.4 ± 2.5 | 9.8 ± 1.3 |
| 6 | 9.4 ± .89 | 8.0 ± 2.3 | 9.2 ± 1.9 |
| 7 | 9.0 ± .70 | 8.0 ± 2.3 | 8.8 ± 2.3 |
| 8 | 9.0 ± .70 | 7.8 ± 2.2 | 8.8 ± 2.3 |
| 9 | 9.0 ± .70 | 7.8 ± 2.2 | 8.8 ± 2.3 |
| 10 | 8.8 ± 1.0 | 7.6 ± 2.2 | 8.8 ± 2.3 |
| 13 | 8.6 ± .89 | 7.2 ± 1.9 | 8.6 ± 2.0 |
| 14 | 8.6 ± .89 | 7.2 ± 1.9 | 8.2 ± 2.2 |
| 15 | 8.2 ± 1.3 | 7.0 ± 1.8 | 8.2 ± 2.2 |
| 16 | 7.7 ± 1.0 | 6.8 ± 1.6 | 8.0 ± 2.3 |
| 17 | 7.4 ± 1.1 | 6.6 ± 1.5 | 8.0 ± 2.3 |
| 20 | 7.2 ± 1.0 | 6.6 ± 1.5 | 7.8 ± 2.0 |
| 21 | 7.0 ± 1.0 | 6.6 ± 1.5 | 7.8 ± 2.0 |
| 22 | 7.0 ± 1.0 | 6.6 ± 1.5 | 7.2 ± 2.5 |
| 23 | 6.8 ± 1.3 | 6.6 ± 1.5 | 7.2 ± 2.5 |
| 24 | 6.4 ± 1.8 | 6.2 ± 1.2 | 6.6 ± 2.1 |
| 27 | 6.0 ± 1.4 | 5.8 ± 1.3 | 5.7 ± 1.9 |
| 28 | 5.7 ± 1.2 | 5.8 ± 1.3 | 5.7 ± 1.9 |
| 29 | 5.2 ± 1.3 | 5.5 ± 1.1 | 5.7 ± 1.9 |
| 30 | 5.2 ± 1.3 | 5.3 ± 0.9 | 5.7 ± 1.9 |

Results of 24 March 1980: Effects of flow in interpreting PCB induced mortality.

Container modifications were designed (see Methods, 4.3) to keep larger amounts of PCB in solution. In addition, the extremely low flow of 1 ml hr was eliminated as a variable which might effect the planktonic Diaptomus.

Survival at 3.43 µg $l^{-1}$ and 20 µg $l^{-1}$ dichlorobiphenyl exceed that in the control (Table 24). When static tests were run in zero flow, survivorship at 20 µg $l^{-1}$ PCB also exceeded that in the control (0 µg $l^{-1}$ PCB with flow or water replacement every 70 hr). Very little difference existed between the experimental (PCB treatment) and control data (Figure 14).

We must conclude that Diaptomus (males) do not show obvious mortality when grown in low concentrations (34-20 µg $l^{-1}$) of dichlorobiphenyl under continuous flow. Static test (no flow) results indicate either that flow tends to increase mortality or that static conditions result in less PCB in solution, probably the latter. Clearly a lack of evidence does not implicate 2,2'PCB with chronic effects of zooplankton. Likely the zooplankton are more important, on an ecosystem basis, in the bioconcentration of PCB's, increasing the body burden in the important food fishes of Lake Huron, than they are as direct recipients of PCB induced maladys.

TABLE 24. NUMBER OF SURVIVING, STARVED *DIAPTOMUS* (MALES) IN FLASKS EXPOSED TO CONCENTRATIONS (3.43 µg $l^{-1}$ AND 20 µg $l^{-1}$) OF 2,2' DICHLOROBIPHENYL, BEGINNING 24 MARCH, 1980

| Day | Control | 3.43 µg $l^{-1}$ | 20 µg $l^{-1}$ | 20 µg $l^{-1}$ (no flow) |
|---|---|---|---|---|
| 0 | 10.2 | 10.0 | 10.0 | 10.0 |
| 1 | 10.0 | 9.8 | 10.2 | 10.0 |
| 2 | 9.8 | 9.6 | 9.8 | 10.0 |
| 3 | 9.5 | 9.2 | 9.8 | 10.0 |
| 4 | 8.6 | 9.2 | 9.6 | 10.0 |
| 7 | 8.4 | 8.6 | 9.0 | 9.5 |
| 8 | 7.8 | 8.8 | 8.2 | 9.0 |
| 9 | 7.6 | 7.6 | 8.2 | 8.5 |
| 10 | 7.2 | 7.0 | 7.6 | 8.0 |
| 11 | 6.6 | 7.0 | 7.4 | 8.0 |
| 14 | 0.0 | 5.2 | 0.4 | 3.5 |

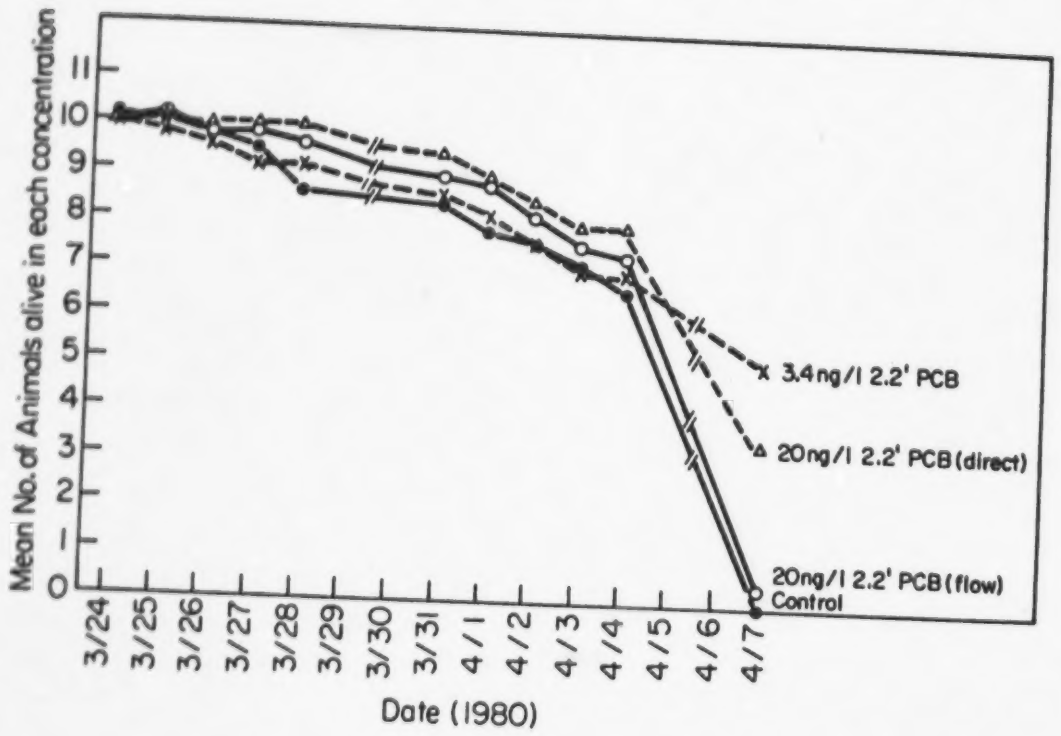


Figure 14. Number of surviving Diaptomus in controls and experimental exposures (3.4 and 20.0 ng $l^{-1}$) over time (days), in flow through experiments.

## 5.4 INHIBITION OF GRAZING AS AN ALGAL CONTROL

### Purpose

Grazing controls the productivity of nannoplankton in oligotrophic Lake Huron (McNaught et al., 1980). The eutrophic waters of Lake Huron are characterized by phytoplankton assemblages dominated by green and bluegreen algae, with the diatoms of importance during the spring. This characteristic applies to Saginaw Bay (Stoermer, unpub. data), as well as the Western Basin of Lake Erie (Munawar and Munawar, 1976). We thus proposed to study grazing in these eutrophic waters, first to see if it was inhibited, and secondly, to see what might be responsible for inhibition if it occurred.

### Concepts of Zooplankton Ingestion

Curvilinear functions have been used to describe the relationship between ingestion rate of planktonic herbivores and their algal foods. Typically ingestion increases linearity or curvilinearity until a critical food concentration has been reached (Rigler, 1961). This food concentration value, which describes the onset of saturation feeding, has been of particular interest. Call the food concentration at saturation, it varies with environment; in freshwater it has been characterized at about $10^5$ cells $ml^{-1}$ (McMahon and Rigler, 1963), but may occur as low as $4 \times 10^1$ cells $ml^{-1}$ in the sea (Frost, 1975).

Typically in open water environments of Lake Huron, feeding does not saturate, and ingestion increases linearily with food concentration (McNaught et al., 1980). Thus it was of interest to see if zooplankton ingestion saturated in Saginaw Bay.

### Zooplankton Uptake Curves (Ingestion vs. Concentration) for Saginaw Bay

Zooplankton ingestion was determined for the eleven species and life

stages of zooplankton which dominante in Saginaw Bay (Table 25). Diaptomus (adults) and Eurytemora (adults) ingested large amounts of nannoplankton on occasion, but these organisms are not abundant. The high ingestion rates for copepod nauplii, Cyclops vernalis, and Daphnia retrocurva are significant, since these organisms are often dominant. These ingestion rates have then been compared to estimates of nannoplankton biomass made from phytoplankton collections on Saginaw Bay (Stoermer, pers. comm.). These biomass estimates are listed in Table 26.

Four species or forms of zooplankton which were important in terms of biomass, or less abundant but present over much of the summer, were selected for comparison with their feeding behavior in the open lake (Figures 15A - D). Copepod nauplii (Δ) and the cladocerans Eubosmina coregoni (O) and Chydorus sphaericus (O) were common in the Bay; Diaptomus adults (Δ) were less common, but important as progenitors of the most vital grazers, the nauplii.

These four grazers all showed intense inhibition of feeding in Saginaw Bay. That is, their uptake curves are characterized by saturation at low food concentrations; in effect these animals were consuming very little nannoplankton, as compared to their feeding behavior in the open lake. When the uptake curves were drawn at an expanded scale, it was observed that saturation occurred about 175 $mgCm^{-3}$ for all four species.

When compared to ingestion-concentration curves for these organisms in the open lake (solid lines, Figure 15 A-D), we find in the cases of Diaptomus, Eubosmina, and Chydorus that their uptake curves lie along the baseline for ingestion of nannoplankton. Thecurve for nauplii shows an increase to 175 $mgCm^{-3}$, but is only visible because of the expanded scale of that graph. Zooplankton ingestion is severely inhibited in Saginaw Bay. How can one mea-

TABLE 25. INGESTION RATE (mgC $ind^{-1}$ $d^{-1}$ x $10^{-6}$) FOR MAJOR SPECIES WITH REGARD TO NANNOPLANKTON RESOURCE CONCENTRATION. SAGINAW BAY, 1976

| Date | Nannoplankton $mgCm^{-3}$ | Ingestion Rate: Diaptomus (A) | Diaptomus (C) | Cyclops vernalis | Mesocylops | Eurytemora | Nauplii |
|---|---|---|---|---|---|---|---|
| 2 July 76 | 72.0 | --- | --- | 55.3 | 41.5 | --- | 12.1 |
| 11 July 76 | 85.0 | 32.6 | 12.2 | 28.6 | 36.7 | 44.9 | 28.6 |
| 9 Oct. 76 | 177.0 | 866. | 169.9 | 203.9 | 140.1 | 437.5 | 212.4 |
| 4 June 76 | 250.7 | 162.4 | 108.3 | --- | --- | 210.6 | --- |
| 3 Sept. 76 | 1032. | 123.8 | --- | 74.3 | 123.8 | 277.2 | 148.6 |

| | | Bosmina longirostris | Eubosmina coregoni | Ceriodaphnia | Chydorus sphaericus | Daphnia retrocurva |
|---|---|---|---|---|---|---|
| 2 July 76 | 72.0 | --- | 69.1 | --- | 43.2 | --- |
| 11 July 76 | 85.0 | 22.4 | 26.5 | 44.9 | 18.4 | 36.7 |
| 9 Oct. 76 | 177.0 | 123.2 | 101.9 | 152.0 | 144.4 | 106.2 |
| 4 June 76 | 250.7 | 48.1 | 78.2 | --- | --- | 258.7 |
| 3 Sept. 76 | 1032. | --- | 49.5 | --- | 123.8 | 74.3 |

TABLE 26. COMPARISON FOOD RESOURCES IN SAGINAW BAY AND OPEN WATERS OF LAKE HURON

| Characteristic | Date | Saginaw Bay | Open Lake |
|---|---|---|---|
| %BG (vol) | 4 June | 0.3 | 0.3 |
| | 11 July | 13.9 | 8.0 |
| | 3 Sept. | 34.3 | 67.3 |
| | 9 Oct. | 31.6 | 21.1 |
| Volume BG ($\mu^3$) | | | |
| | 4 June | 11156 | 42 |
| | 11 July | 48549 | 5059 |
| | 3 Sept. | 58752 | 58752 |
| | 9 Oct. | 1488 | 12477 |
| Phytoplankton Productivity ($mgCm^{-3}\ hr^{-1}$) | | | |
| | 4 June | 7.9 | 0.7 |
| | 11 July | 53.2 | 10.0 |
| | 3 Sept. | 159.9 | 19.1 |
| | 9 Oct. | 20.4 | 0.5 |
| Size & Species Phytoplankton | | | |
| | 4 June | Fragilaria cr. (LG) | Fragilaria cr. (LG) |
| | 11 July | Chroococcus (SM) | Fragilaria cr. (LG) |
| | 3 Sept. | Anacystis (SM) | Chroococcus(SM); Cyclotella c.(SM) |
| | 9 Oct. | Anacystis(SM); Cyclotella(SM) | Anacystis(SM); Cyclotella c.(SM) |

BG = bluegreens, LG = large, SM = small

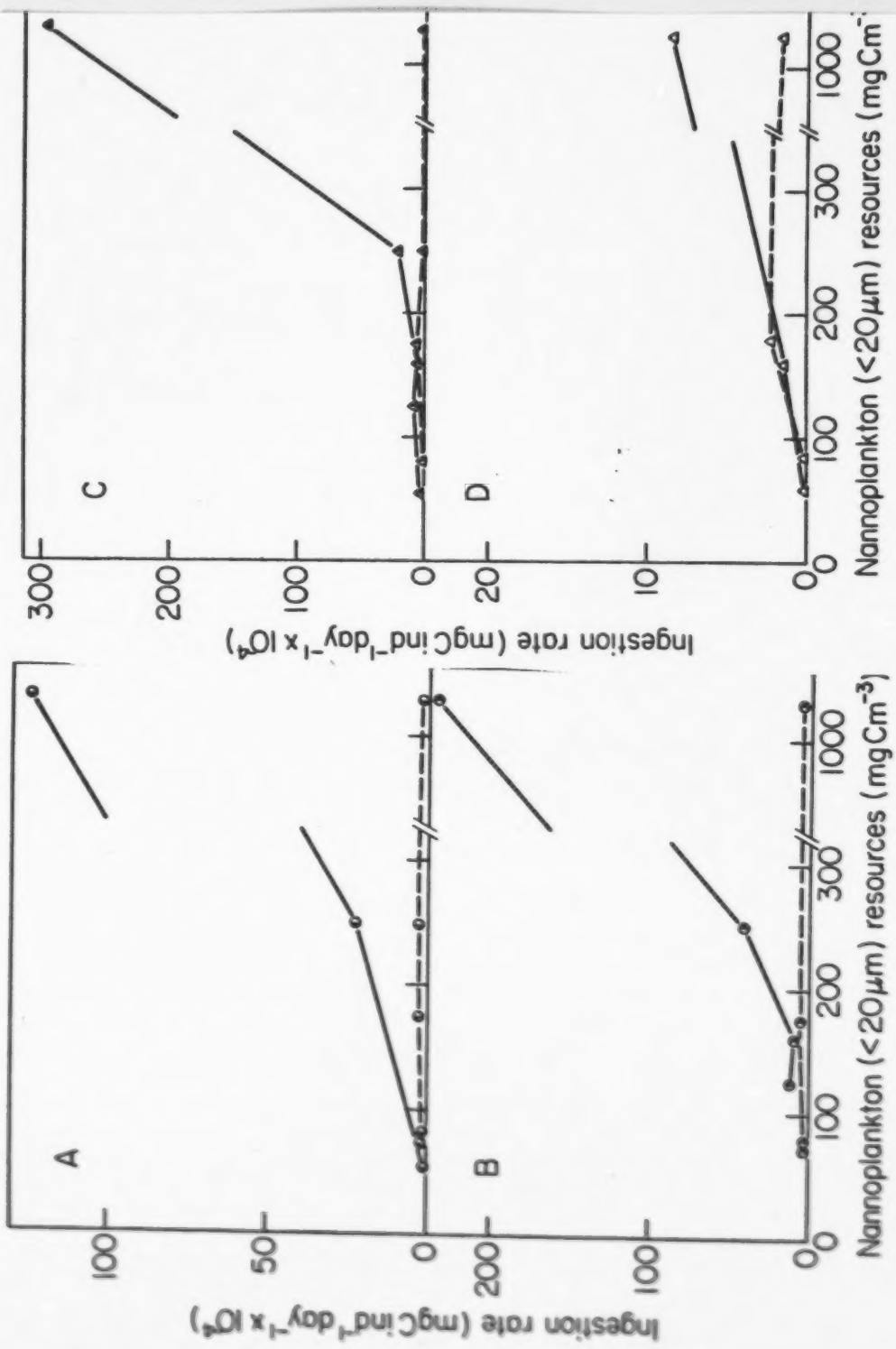


Figure 15. A-D. Ingestion-concentration curves for Eubosmina (o), Chydorus (o), Diaptomus adults (Δ) and nauplii (Δ) in Saginaw Bay (dashed line), as compared to the same ingestion rates as identical concentrations in the open waters of Lake Huron (solid line).

sure inhibition, and what are the causes?

Modelling Inhibition of Ingestion

Inhibition of ingestion was estimated using a double Monod model suggested by D. DiToro of Manhattan College (pers. comm.). Ingestion (I) is described in terms of the half-saturation constant (Km) for ingestion and the food concentration (Co) at the onset of ingestion. Inhibition (Ih) is described in terms of the food concentrations at the onset of inhibition ($C_h$) and the half-saturation constant $(Km_h)_1$ as diagrammed in Figure 16, such that:

$$I = \frac{Ic}{Km + C} - \frac{I_h(C-C_h)}{Km_h\ (C-Ch)}$$

Ingestion of Observed Inhibition in Saginaw Bay

Algal Foods--

The phytoplankton of Saginaw Bay are similar in relative composition to those of the open lake (Table 26). The waters of the Bay are dominated by diatoms (D) during the spring. These give way to greens during the summertime, although during late summer the greens occur in almost equal biomass as the bluegreens (about 30% by volume), as shown in Figure 17. Thus relative composition is not the only key to our story.

Our overwhelming feature separating the waters of the Bay from those of the open lake is the large biomass of bluegreens. For example, during June they are 265 times more abundant than in the open lake. Zooplankton in Lake Huron prefer not to ingest sheathed algae (McNaught et al., 1980). This great abundance of bluegreens and greens in Saginaw Bay is reflected in the rates of productivity found there as opposed to the open lake (Table 26).

Levels of Relative Inhibition of Ingestion--

The herbivorous crustaceans of Saginaw Bay are inhibited from ingesting

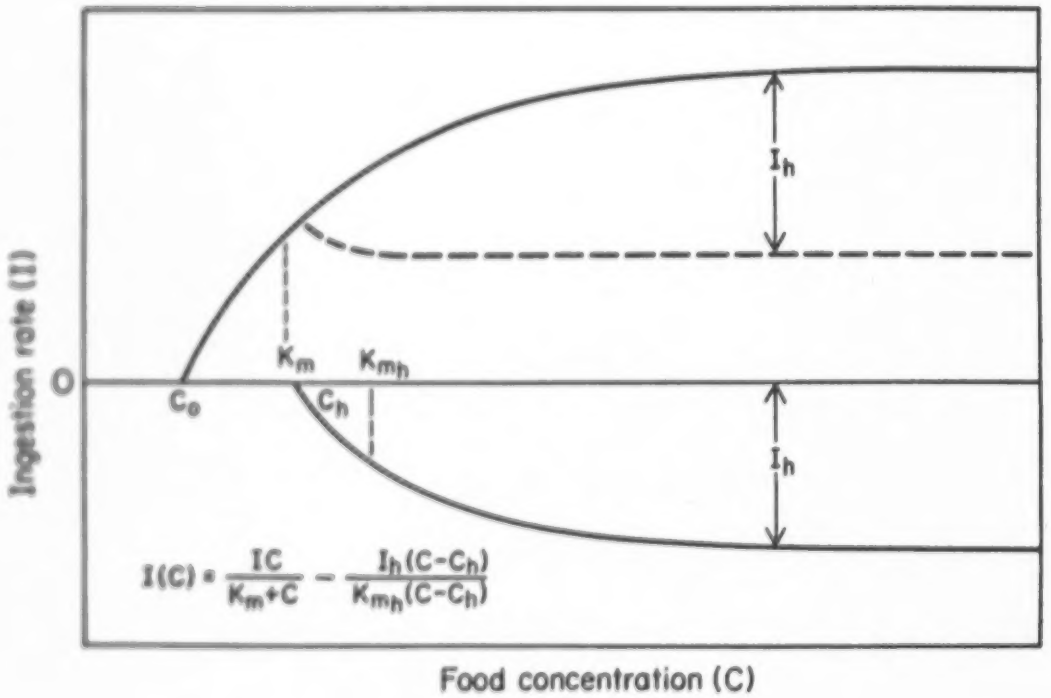


Figure 16. Model for inhibition of ingestion (I); symbols in text.

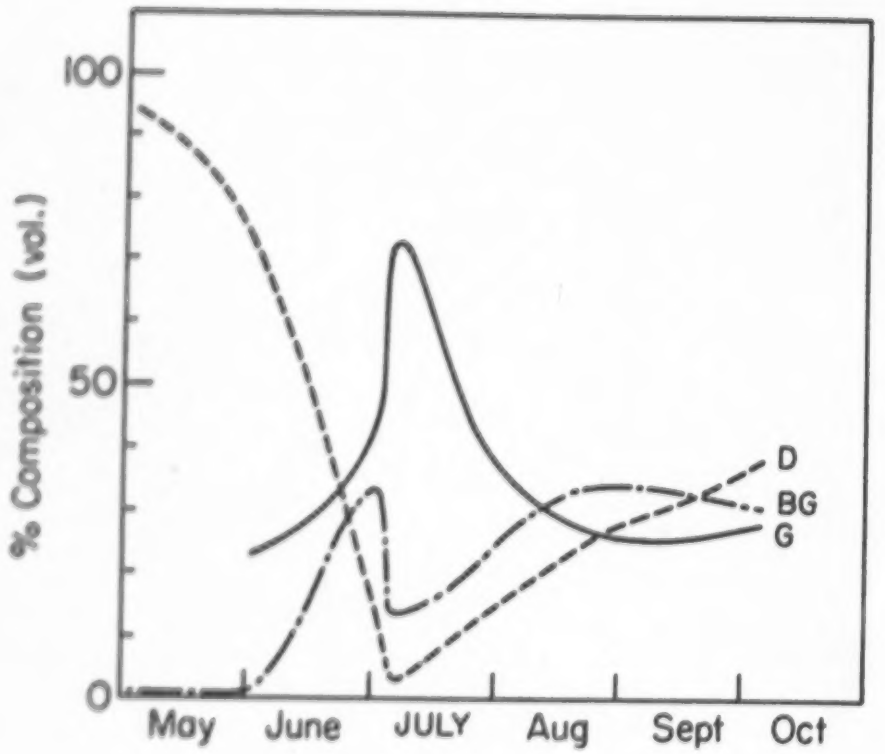


Figure 17. Relative composition (by volume) of phytoplankton community of Saginaw Bay, including diatoms (D), green (G) and blue-green (BG) algae.

TABLE 27. ESTIMATES OF RELATIVE INHIBITION ($I_h$) OF INGESTION BY CRUSTACEANS IN SAGINAW BAY DURING 1976 $\frac{}{I\ lake}$

| Date | Species | $I_h/I$ lake (%) |
|---|---|---|
| 4 June | Diaptomus (A) | 88.1 |
| | Eubosmina coregoni | 96.4 |
| 11 July | Eubosmina coregoni | 20.6 |
| | Chydorus sphaericus | 97.6 |
| 3 Sept. | Diaptomus (A) | 99.6 |
| | Eubosmina coregoni | 99.4 |
| | Chydorus sphaericus | 99.5 |
| 9 Oct. | Chydorus sphaericus | 93.2 |

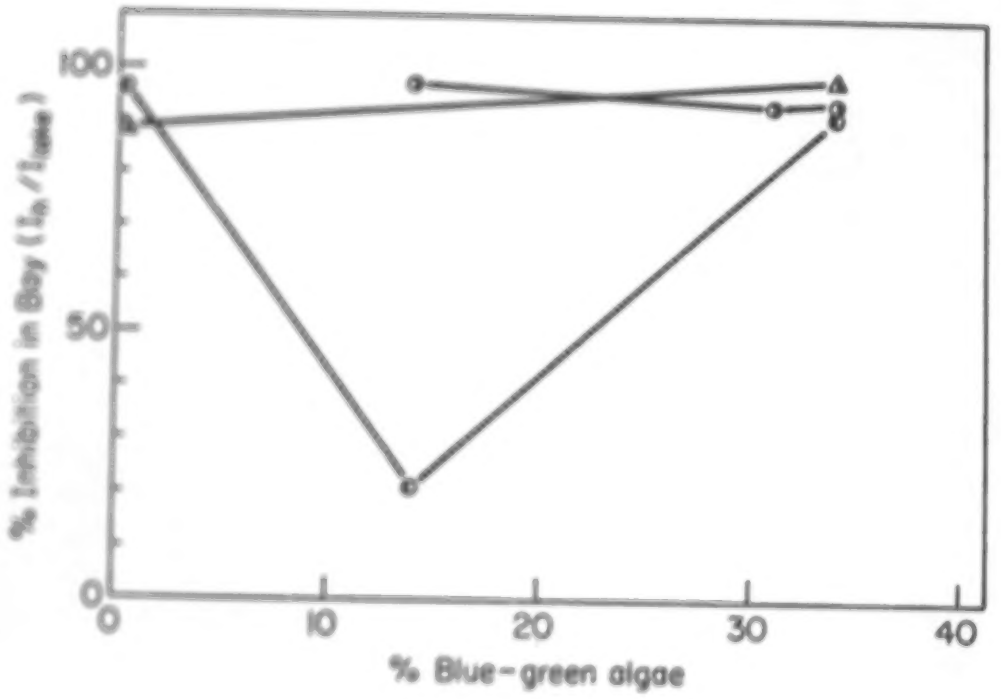


Figure 18. Percentage inhibition by Eubosmina (o), Chydorus (•), and Diaptomus (Δ) as a function of the percentage composition of blue-green algae in Saginaw Bay.

food at levels of 20.6 to 99.6% compared to the organisms of the open lake (Table 27). The mean inhibition of ingestion in Saginaw Bay is 86.8%, that is, these crustacenas of only consuming 13.2% of the amount expected, based on linear uptake to food concentrations of 100 $mgCm^{-3}$. This is a striking finding. Biological control of nuisance blooms of algae is unlikely if only 13% of the optimal grazing activity is possible.

Interpretation of this almost total inhibition of grazing is difficult. The obvious comparison to be made, according to the grazing literature, would be between inhibition and the percentage composition of bluegreen algae (Figure 18). Surprisingly, there is little relationship, chiefly because inhibition was only observed at levels below 88% once. Diaptomus and Chydorus were inhibited more than 88% at all levels of bluegreen composition, while Eubosmina was inhibited only 20.6% at an intermediate bluegreen relative biomass. We must conclude tht grazing is severely limited in Saginaw Bay by factors other than bluegreen abundance. The high levels of other natural and man-made compounds in the water must be investigated.

Zooplankton Uptake Curves for Lake Erie

Algal Foods--

Levels of algal carbon for Western Lake Erie were calculated from the data presented by Munawar and Munawar (1976). Nannoplankton populations are dominated in April by diatoms (79%), in May by cryptomonads (18%), in early July by diatoms (23%) and cryptomonads (22%), in late July by Ceratium (20.5%) and Pediastrum (19.5%), and in September by diatoms (33%) and bluegreen algae (20%).

Levels of Relative Inhibition of Ingestion--

The herbivorous crustaceans of Western Lake Erie are not inhibited from

feeding, based on comparison with the linear uptake curves for the zooplankton of Lake Huron. Their ingestion rates vary from 63.4 x $10^{-6}$ mgC $ind^{-1}$ $day^{-1}$ for Chydorus at intermediate nannoplankton carbon (800 $mgCm^{-3}$) to 3010 for Daphnia galeata at a higher food level (1350) Table 28. The graphs comparing the various uptake-concentration relationships for Lake Erie are clear evidence that these herbivores equal and even outperform their counterparts in the open waters of Lake Huron.

Eubosmina (Figure 19A) exhibited equivalent or higher ingestion rates than the same species in Lake Huron; even though Lake Erie is rich in nannoplankton, with almost twice the biomass as the open waters of Lake Huron, the rates above food concentrations of 1400 $mgCm^{-3}$ are only slightly below the projected curve for Lake Huron. The same is true for Chydorus (Figure 19B). Ingestion rates at 1424 and 1785 $mgCm^{-3}$ as nannoplankton lie only slightly below the projected curve for Lake Huron. Since the nannoplankton biomass does not reach 1800 $mgCm^{-3}$ in mesotrophic Lake Huron, we cannot realistically calculate inhibition levels for Lake Erie. If we were to estimate a level of inhibition for Chydorus in Lake Erie relative to the open waters of Lake Huron, we would find that Chydorus was only inhibited about 17%, nothing approaching the levels observed for the same species in Saginaw Bay.

Similar conclusions are evident for Diaptomus (adults) and nauplii. The nauplii are the most important nannoplankton grazers in the Great Lakes. Yet in Western Lake Erie their ingestion rates exceed the levels found in the open waters of Lake Huron. We must conclude that they control nannoplankton growth rather effectively in Lake Erie. The same was true for Diaptomus. Ingestion levels at a food concentration of 1300 $mgCm^{-3}$ are about the same

TABLE 28. INGESTION RATE (mgC $ind^{-1}$ $d^{-1}$ x $10^6$) FOR MAJOR SPECIES WITH REGARD TO NANNOPLANKTON RESOURCES LAKE ERIE, STATION , 1977 (A = ADULT, C = COPEPODITE)

| Date | Nannoplankton $mgCm^{-3}$ | Diaptomus (A) | Diaptomus (C) | Ingestion Rate Cyclops vern. | Eurytemora | Nauplius |
|---|---|---|---|---|---|---|
| 19 April 1977 | 800 | 52.0 | 54.7 | 230.4 | - | 73.9 |
| 23 May 1977 | 1424 | 411.8 | - | 150.3 | 539.9 | 95.7 |
| 7 July 1977 | 1000 | 348.0 | - | 144.0 | - | 31.0 |
| 26 July 1977 | 1350 | 1263.6 | 680.4 | 216.0 | - | 178.0 |
| 15 Sept. 1977 | 1785 | 428.4 | 244.0 | 327.0 | - | 110.6 |

| | Daphnia galeata (A) | Bosmina longirostris | Eubosmina coregoni | Cyclopoid cops. | Chydorus | Daphnia retrocurva | Cyclops bicuspidatus (A) |
|---|---|---|---|---|---|---|---|
| 19 April 1977 | 960.0 | - | - | 25.9 | 63.4 | - | 334.0 |
| 23 May 1977 | - | 210.2 | 220.4 | 28.5 | 261.4 | 710.9 | 193.0 |
| 7 July 1977 | 2472. | 264.0 | 61.0 | 44.0 | 636.0 | 1888.0 | 300.0 |
| 26 July 1977 | 3010. | - | 745.0 | 388.8 | - | 1927.8 | - |
| 15 Sept. 1977 | - | - | 178.5 | 216.3 | 278.4 | 473.0 | - |

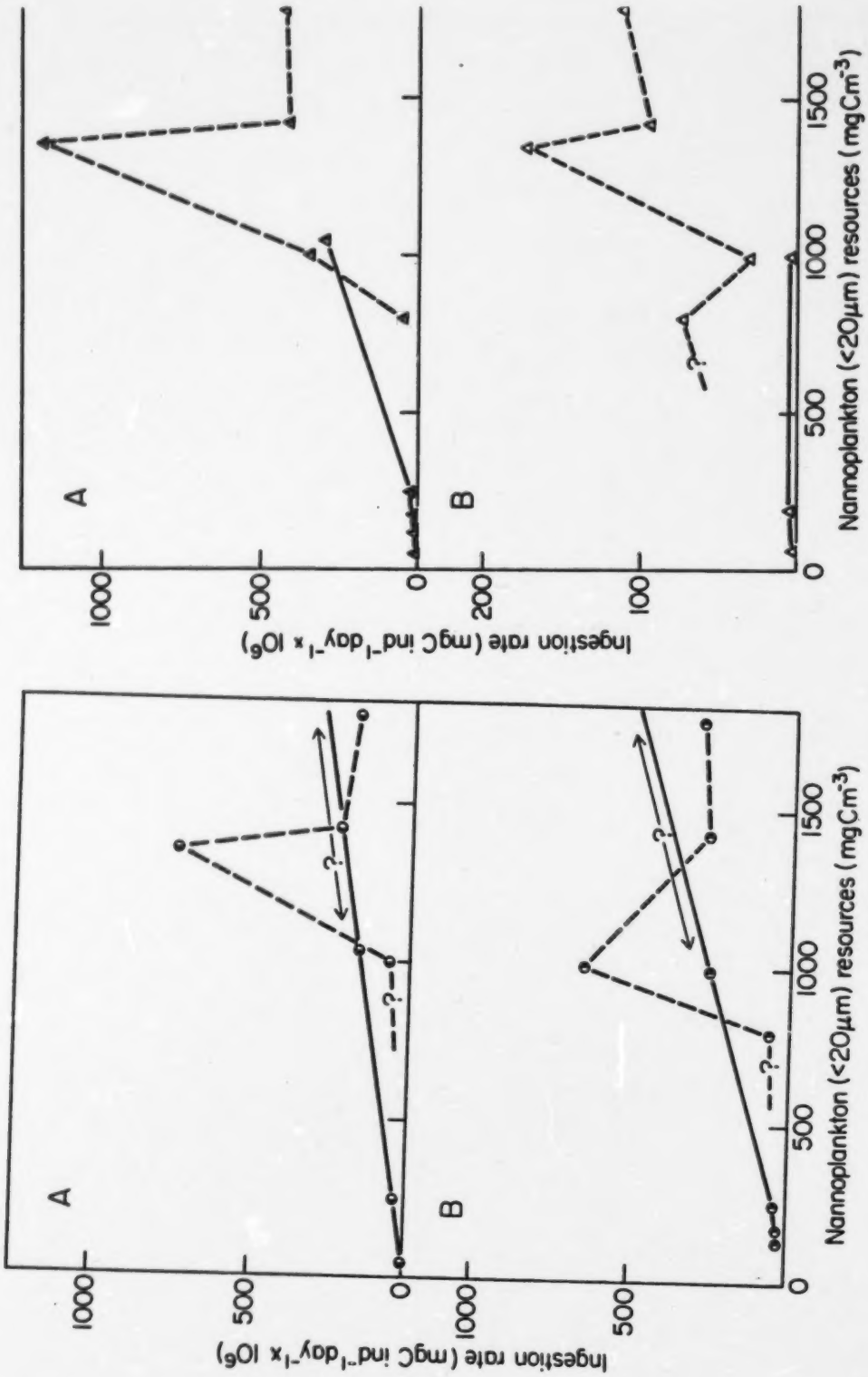


Figure 19. A-D. Ingestion-concentration curves for Eudosmina (o), Chydorus (o), Diaptomus adults (Δ) and nauplii (Δ) in Western Lake Erie (dashed line) compared to the same ingestion rates at identical concentrations in the open waters of Lake Huron (solid line).

as those measured in Lake Huron. If a calculation of inhibition were made it would be positive (stimulation).

These findings concerning bioregulation of phytoplankton growth in Lake Erie are both unexpected and stimulating. Why has the Lake Erie ecosystem responded in this fashion? Why is grazing phytoplankton effective in Lake Erie and not in Saginaw Bay?

Comparative Assimilation Rates on Total Phytoplankton

The growth of any zooplankton population is equivalent to its assimilation of food, and not simply to the food ingested. In the open waters of Lake Huron food is scarce. The zooplankton grazed over 100% of nannoplankton productivity from May through August. Of the scarce nannoplankton ingested, the major herbivores assimilated 93.2% (McNaught et al., 1980). However, both Saginaw Bay and Western Lake Erie appear to be food rich environments, where herbivores could feed rapidly but tolerate a relatively low efficiency. In one case, Saginaw Bay, populations of zooplankton did not ingest much, and their assimilation rates may help interpret what was happening.

Assimilation rates are difficult to measure in the field. We followed classical techniques (Sorokin, 1968) of determining ingestion using a short feeding period (15 min). Then we feed a duplicate batch for a long period, following which the zooplankters guts were cleared of tagged food for 4 hr. Relative assimilation can be defined as:

$$\% \text{ assimilation} = \frac{\text{ingestion rate determined with defication possible}}{\text{short-term ingestion rate}}$$

Lake Erie zooplankters ingested large amounts of food from an environment productive of diatoms and cryptomonads, with some greens and bluegreens. Their intake could have been predicted by extending the linear uptake-concen-

tration curve determined for the zooplankton of the open waters of Lake Huron. However, from this large food supply they assimilated only 8.4% of the food ingested (Table 29). Assimilation was highest (21.3%) during lake May when small cryptomonads are typically abundant.

Saginaw Bay crustaceans live in an eutrophic environment with a smaller algal standing crop than Western Lake Erie (Table 26). While their intake of nannoplankton was inhibited 87% below that in the open lake, their assimilation values were higher, with a mean of 14.5% and a high of 45.7% for June (Table 30). In June the phytoplankton of the Bay was dominated by diatoms. We cannot determine if this higher level of food assimilation helps defray the costs of low ingestion. Certainly it is part of the answer to balancing the equation for consumption. Alternate foods as detritus probably account for the remainder of the answer to this stimulating question.

TABLE 29. ASSIMILATION EFFICIENCY (%) ON TOTAL PHYTOPLANKTON FOR LAKE ERIE ZOOPLANKTON COMMUNITY DURING 1977

| Species | 19 April | 23 May | 7 June | 26 July | 15 Sept. |
|---|---|---|---|---|---|
| Cladocera | | | | | |
| Daphnia galeata | 16.7 | - | 1.2 | 9.9 | - |
| Daphnia retrocurva | - | 64.0 | 0.6 | 3.8 | 14.7 |
| Chydorus sphaericus | - | 11.1 | 0 | - | 0 |
| Bosmina longirostris | - | 8.3 | - | - | - |
| Eubosmina coregoni | - | 2.7 | 5.4 | 2.8 | 10.6 |
| Leptodora kindtii | - | - | 2.1 | 6.7 | 0 |
| Copepoda | | | | | |
| Nauplii | 0 | 0 | 0 | 3.3 | 0 |
| Calanoid copepodites | 25.0 | - | - | 6.8 | 16.2 |
| Diaptomus (A) | 4.8 | - | - | 5.2 | 12.5 |
| Limnocalanus macrurus (A) | 3.5 | - | - | - | - |
| Epischura lacustris | - | - | - | 5.8 | - |
| Eurytemora affinis | - | 27.5 | - | 34.6 | - |
| Cyclopoid copepodites | 0 | 57.1 | 0 | 0 | 0 |
| Cyclops bicuspidatus | 0 | 6.8 | 1.5 | - | - |
| Cyclops vernalis | 0 | 14.3 | 2.4 | 4.0 | 0 |
| Mesocyclops edax | 0 | - | - | - | - |
| Monthly Mean | 5.6 | 21.3 | 1.6 | 7.5 | 6.0 |
| Grand Mean = 8.4 | | | | | |

TABLE 30. ASSIMILATION EFFICIENCY (%) ON TOTAL PHYTOPLANKTON FOR SAGINAW BAY ZOOPLANKTON COMMUNITY DURING 1976

| Species | 5 June | 11 July | 29 July | 4 Sept. | 10 Oct. |
|---|---|---|---|---|---|
| Cladocera | | | | | |
| Daphnia retrocurva | 60.0 | 8.8 | 5.8 | 0 | 14.9 |
| Chydorus sphaericus | 65.0 | 9.6 | 0 | 0 | - |
| Eubosmina coregoni | 38.8 | 13.0 | 0 | 0 | 0 |
| Bosmina longirostris | 33.3 | 9.7 | - | - | 0 |
| Alona spp. | | | | | |
| Copepoda | | | | | |
| Nauplii | 0 | 0 | 0 | 0 | 0 |
| Calanoid copepodites | - | - | - | 0 | - |
| Eurytemora affinis | 100.0 | 12.8 | 14.4 | 17.8 | 68.0 |
| Diaptomus (A) | 30.0 | 16.6 | 3.6 | 0 | - |
| Cyclopoid copepodites | 30.3 | - | - | 0 | - |
| Cyclops bicuspidatus | 62.5 | - | - | - | - |
| Cyclops vernalis | 37.7 | 8.0 | 4.1 | 0 | - |
| Mesocylops edax | - | - | - | 0 | 1.8 |
| Tropocylops prasinus | - | 0 | 0 | 0 | 5.0 |
| Monthly Mean | 45.7 | 8.7 | 3.5 | 1.6 | 12.8 |
| Grand Mean = 14.5 | | | | | |

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